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Pollution prevention in wastewater networks: Development of a biological early warning device

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ABSTRACT

A biological early warning system (EWS) was developed to screen wastewater containing nitrification inhibitors and identify nitrifying bacteria activity reduction without relying on absolute values of sensor signals. To do so, numerous sensors were evaluated using a tiered approach to aid the analysis and made it easier to convey the current state of the technology. The research then produced a framework for the development of an EWS and the applicability of sensors to the wastewater matrix. The research identified a need for the development of a strategy and guidance that can help in the prevention and detection of nitrification inhibitors. Initial tests focussed on sewer biofilm N_2O emissions, however, despite average nitrification rates of 19.5 g-NH_4^+-N.m^{-2}.d^{-1} the response was unreliable due to inadequate control. To address this, a circulating floating bed biofilm reactor (CFBBR) was designed as a sidestream. The CFBBR biofilm's toxicity response was compared to the sewer biofilm, a 2850 mg.L^{-1} MLSS culture and a 10.5 mg.L^{-1} MLSS culture (with equivalent biomass concentration to the CFBBR biofilm). The cultures responded differently with an inhibitory effect scale of Cu^{2+} > ATU > Ni^{2+} > Cr^{6+} for CFBBR biofilm, ATU > Cu^{2+} > Ni^{2+} > Cr^{6+} for 2850 mg L^{-1} MLSS, ATU > Ni^{2+} > Cr^{6+} > Cu^{2+} for 10.5 mg.L^{-1} MLSS and ATU > Cu^{2+} > Cr^{6+} > Ni^{2+} for sewer biofilm. This was firstly attributed to suspended growth nitrification stimulation by Cu^{2+} doses up to ~45 mg.L^{-1} resulting in a lower inhibitory effect. Secondly, very high Cr^{6+} and Ni^{2+} doses were required for biofilm nitrification inhibition, due to diffusion limitations and slow transport through cell membranes. The CFBBR biofilm response to heavy metals was characterised through N_2O and CO_2 spikes and a post shock emissions recovery period was observed with the trend Ni^{2+} > Cr^{6+} > Cu^{2+}. A 10 minute hydraulic retention time allowed quick detection and steady state nitrification rates of 0.4 g-NH_4^+-N.m^{-2}.d^{-1} despite high organic loading rates. Additionally, a suspended growth based monitor (Nitritox) was assessed as an inlet works toxicity detector. Incorporation of a Nitritox with a CFBBR based sewer monitor offered increased robustness over a CFBBR only system and was shown to be viable system in catchments >200,000 population equivalent. This information is useful to water utilities so that they can plan for and experiment with upset early warning protocols. It is also useful to manufacturers as they can determine product performance needs.

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DEVELOPMENT OF AN IN-SEWER EARLY WARNING SYSTEM

ABBREVIATIONS

AC	Alternating current
AH	Aerobic heterotrophs
AMO	Ammonium mono-oxygenase
ANOVA	Analysis of variance
AOB	Ammonia oxidizing bacteria
APHA	American Public Health Association
AS	Activated sludge
ASP	Activated sludge plant
ASV	Anodic stripping voltammetry
ATU	Allylthiourea
BOD	Biochemical oxygen demand
CF	Correction factor
CFBBR	Circulating floating bed biofilm reactor
COD	Chemical oxygen demand
CSS	Combined sewer system
CTM	Continuous toxicity monitor
CUSUM	Cumulative sum
DC	Direct current
DO	Dissolved oxygen
DTA	Direct toxicity assessment
DWF	Dry weather flow
$EC_{50\,/\,75}$	Effective concentration 50 / 75
EPS	Extracellular polymeric substances
EPSRC	Engineering and physical science research council
EWS	Early warning system
FCM	Flow Cytometry
FFT	Flow to full treatment
FIA	Flow injection analysis
FOG	Fats, oils and greases
FSS	Fixed suspended solids

GPRS	General packet radio service
GSM	Global system for mobile communications
HAO	Hydroxylamine oxyreductase
HDN	Heterotrophic denitrifying bacteria
HRT	Hydraulic retention time
IP68	Ingress protection 68
ISE	Ion selective electrode
ISO	International Standards Organisation
LAN	Local area network
MLSS	Mixed liquor suspended solids
NAP	Periplasmatic nitrate reductase
NAR	Nitrate reductase
NI	Nitrification inhibition
NOB	Nitrite oxidising bacteria
NOS	Nitrous oxide reductase
NXR	Nitrite oxidoreductase
NR	Nitrification rate
OC	Organic carbon
OUR	Oxygen uptake rate
PE	Population equivalent
PC	Personal computer
RTC	Real time control
SCADA	Supervisory control and data acquisition system
SPS	Sewage pumping station
SRB	Sulphate reducing bacteria
TOC	Total organic carbon
TS	Total solids
TSS	Total suspended solids
USB	Universal serial bus
VOC	Volatile organic carbon
VS	Volatile solids
VSS	Volatile suspended solids

WwTW Wastewater treatment works

NOTATIONS

%	Percentage
°C	Degree Celsius (unit of temperature)
Atm	Standard unit of pressure (101,325 Pa)
C	Concentration
$CaCl_2$	Calcium chloride
Cu^{2+}	Copper (II)
Cr^{6+}	Chromium (VI)
CH_4	Methane
CO_2	Carbon dioxide
d	Day
$FeSO_4.7H_2O$	Iron (II) sulphate heptahydrate
g	Gram
hr	Hours
H_2S	Hydrogen sulphide
KCl	Potassium chloride
kg	Kilogram
KH_2PO_4	Monopotassium phosphate
KNO_3	Potassium nitrate
L	Litre
m	Metre
m^2	Square metre
m^3	Cubic metre
mA	Milliamp
mg	Milligrams
$mg.L^{-1}$	Milligrams per litre
min	Minute
ml	Millilitres
mm	Millimetre

mol	Mole
mS	Millisiemens
$MgSO_4$	Magnesium sulphate
$MnSO_4.4H_2O$	Manganese sulphate tetrahydrate
N	Nitrogen
N_2O	Nitrous oxide
NaCl	Sodium chloride
$Na_2HPO_4.12H_2O$	Disodium hydrogen orthophosphate dodecahydrate
$NaHCO_3$	Sodium bicarbonate
NH_2OH	Hydroxylamine
NH_4^+/NH_4-N	Ammonium/Ammonium-nitrogen
NH_4Cl	Ammonium chloride
Ni^{2+}	Nickel (II)
NO	Nitric oxide
NO_2^-/NO_2-N	Nitrite/Nitrite-nitrogen
NO_3^-/NO_3-N	Nitrate/Nitrate-nitrogen
pH	Power of hydrogen
ppm	volumetric parts-per-million
R	Reactor
t	Time
V	Volts
v/v	Volume by volume
Q	Flow rate
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate

EXECUTIVE SUMMARY

Illegal discharges of toxicants to the sewer can inhibit biological activity and treatment performance at the wastewater treatment works (WwTW). This can lead to a discharge of partially treated sewage to the environment, resulting in damage to the ecosystem, as well as financial penalties and damage to public perception of all parties involved. Hence, monitoring wastewater toxicity on-line before it reaches the WwTW, could be the missing link in protecting the wastewater treatment process and the environment. This work aimed to test the hypothesis that development of an early warning system (EWS) to screen wastewater containing nitrification inhibitors will allow preventative action to minimise adverse effects to the secondary treatment process (i.e. activated sludge process) at the WwTW.

Biological wastewater treatment processes rely on a healthy microbial community to achieve the required treatment performance, leaving them vulnerable to failures when receiving toxic influent loads. However, despite this, on-line toxicity monitoring of crude sewage (in-sewer or at the WwTW) is rarely implemented as it poses a harsh environment for sensor placement. To effectively warn of acute toxic events, a non-invasive method such as off-gas analysis is preferable due to the reduced risk of sensor fouling when compared to direct toxicity analysis (DTA) techniques. In this study, a biological EWS device was developed focussing on nitrous oxide (N_2O) and carbon dioxide (CO_2) gas emitted by nitrifying bacteria as a stress response. This led to the overall project aim; to characterise the response of nitrifying biofilms to toxicity and establish the suitability of employing the technique as the basis of an in-sewer EWS.

The suitability of monitoring off-gas emissions from the sewer biofilm was assessed using the N-Tox N_2O gas monitor at a sewage pumping station feeding a WwTW with a treatment capacity of 342,000 population equivalent (PE) and on pilot scale sewer under controlled conditions. Variable and unreliable response signals from the N-Tox N_2O gas monitor were observed, potentially due to the low abundance of nitrifying bacteria and low hydraulic retention time (HRT). In order to overcome this limitation, circulating floating bed biofilm reactors (CFBBR) of 7 L volume were designed and bioengineered to sustain a nitrifying biofilm at high organic loading rates as experienced in a genuine sewer. The CFBBRs were operated for 390 days fed with a fresh feed of wastewater at a WwTW accepting domestic and industrial wastewater. Nitrification performance was

allowed to stabilise initially for 180 days. To allow for quick detection of a toxic plug, a 10-minute HRT was set. Average nitrification rates of 0.4 g-NH_4^+-N.m^{-2}.d^{-1} (comparable to reported pilot scale nitrifying biofilms under high organic loading rates) was achieved under steady state, following an initial seed and pre-growth period, giving adequate nitrification activity for N_2O production. Indeed, the N_2O emissions from the CFBBR biofilm were an order of magnitude higher than the sewer pipe wall biofilm allowing toxicity responses to be more easily defined. The response of the CFBBR biofilm to known toxicants including Allylthiourea (ATU), Potassium dichromate, Cupric Sulphate and Nickel Sulphate, was then tested at a range of concentrations, including those determined (in dose response tests) to completely inhibit nitrification of the CFBBR biofilm. All shock events were applied for a period of 2 hours and each condition tested took a month to complete allowing for the biofilm acclimatisation period.

The sensitivity of the CFBBR biofilm (especially the nitrifying community) to the shock load event was assessed in off-line nitrification inhibition assays. The response of the nitrifying communities in the CFBBR biofilm was also compared to the mixed liquor suspended solids (MLSS) communities response and the sewer pipe wall biofilm with respect to inhibition of ammonium removal performance. Biofilms in CFBBR and MLSS systems had similar sensitivity to ATU. There were more sensitive to copper (II) and less sensitive to chromium (VI) and nickel (II). The trend and order of inhibitory effect was Cu^{2+} > ATU > Ni^{2+} > Cr^{6+} for the CFBBR biofilm, ATU > Cu^{2+} > Ni^{2+} > Cr^{6+} for 2850 mg L^{-1} MLSS, ATU > Ni^{2+} > Cr^{6+} > Cu^{2+} for the 10.5 mg L^{-1} MLSS and ATU > Cu^{2+} > Cr^{6+} > Ni^{2+} for the sewer biofilm. Overall, the CFBBR biofilm displayed a suitable sensitivity to nitrification inhibitors, measured as ammonium removal, to allow toxicity to be characterised. The response through gaseous emissions was then assessed.

The CFBBR biofilm responded well to heavy metal toxicity, with a positive correlation between heavy metal concentration and the peak height / intensity of a gaseous emissions spike. As mentioned earlier, the biofilm was particular sensitive to Cu^{2+} toxicity evidenced with steep spikes in N_2O and CO_2 (Figure 1). Post shock, emissions dropped below the overall baseline, and the recovery period length was commensurate to the metal salts used with the overall trend of Cu^{2+} (a minimum of 2.5 hours for

recovery) > Cr^{6+} (a minimum of 2.2 hours recovery) and > Ni^{2+} (minimum of 4.0 hours recovery). It was, however, not possible to characterise the response of the CFBBR biofilm to ATU toxicity through gaseous N_2O and CO_2 emissions.

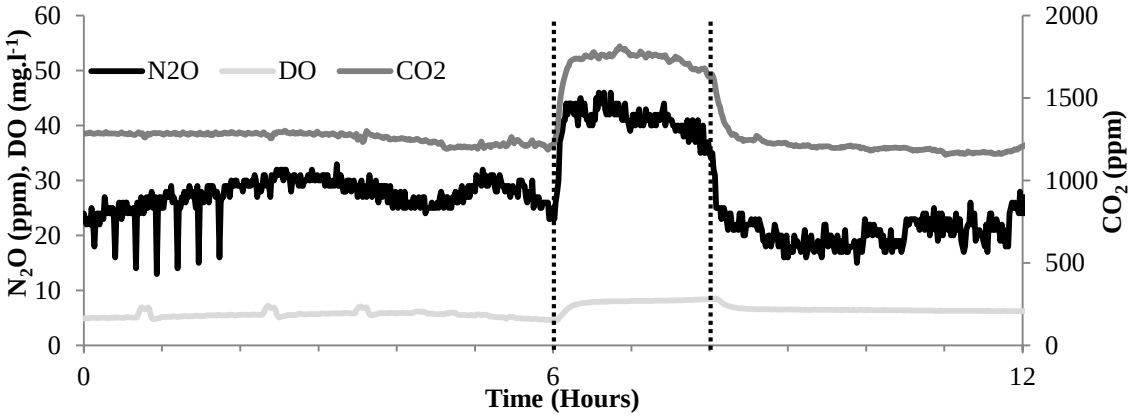


Figure 1 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm. After 6 hours, a 2 hour shock was simulated by adding 96 mg L^{-1} copper (II) to the wastewater (window time delineated by the dotted lines).

Based on the pilot data from the CFBBR experiments, a biological EWS incorporating a CFBBR biofilm was designed and developed for use in sewer catchments. Catchments accepting discharges from high risk industries such as mining, smelting, metallurgical, semiconductor manufacturing, electroplating, tanneries and metal finishing as well as potential landfill leachate carried to the sewer through surface run-off were deemed suitable. Six implementation options were investigated including:

a) **Basic biofilm based EWS;** One CFBBR based system at the inlet works and one in the sewer network. This represents the basic installation to give an in-network early warning and rationalise the response at the inlet works.
b) **Multiple location biofilm based EWS;** One CFBBR based system at the inlet works and two in the sewer network. This system represents an upgrade of system A, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works.
c) **Basic mixed EWS;** One Nitritox at the inlet works and one CFBBR based systems in the sewer network. This system represents an upgrade of system A, whereby an early warning can be provided in the network and rationalisation of that response with a percentage nitrification inhibition calculation by the Nitritox at the inlet.

d) **Multiple location mixed EWS;** One Nitritox at the inlet works and two CFBBR based systems in the sewer network. This system represents an upgrade of system C, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works.

e) **Basic CO_2 mixed EWS;** One Nitritox at the inlet works and one CFBBR based system (monitoring CO_2 only) in the sewer network. Findings from this study suggest a biofilm based EWS can respond to the same toxicant spectrum employing only CO_2 monitoring and omitting the N-Tox N_2O monitor. This system was included to compare against system C.

f) **Multiple location CO_2 mixed EWS;** One Nitritox at the inlet works and two CFBBR based systems (monitoring CO_2 only) in the sewer network. This system represents an upgrade of system E, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works. This system was included to compare against system D.

Each option was assessed based on the risks, benefits and the whole life costs. From the technical risk analysis and whole life costs, it was found that a typical installation will include a CFBBR unit at a sewage pumping station within the sewer network, and another unit at the WwTW inlet to rationalise the response of the nitrifying biofilm. Each CFBBR unit will include a CFBBR, N-Tox, CO_2 monitor, feed pump, controlling computer, internet connection and an autosampler (Figure 2).

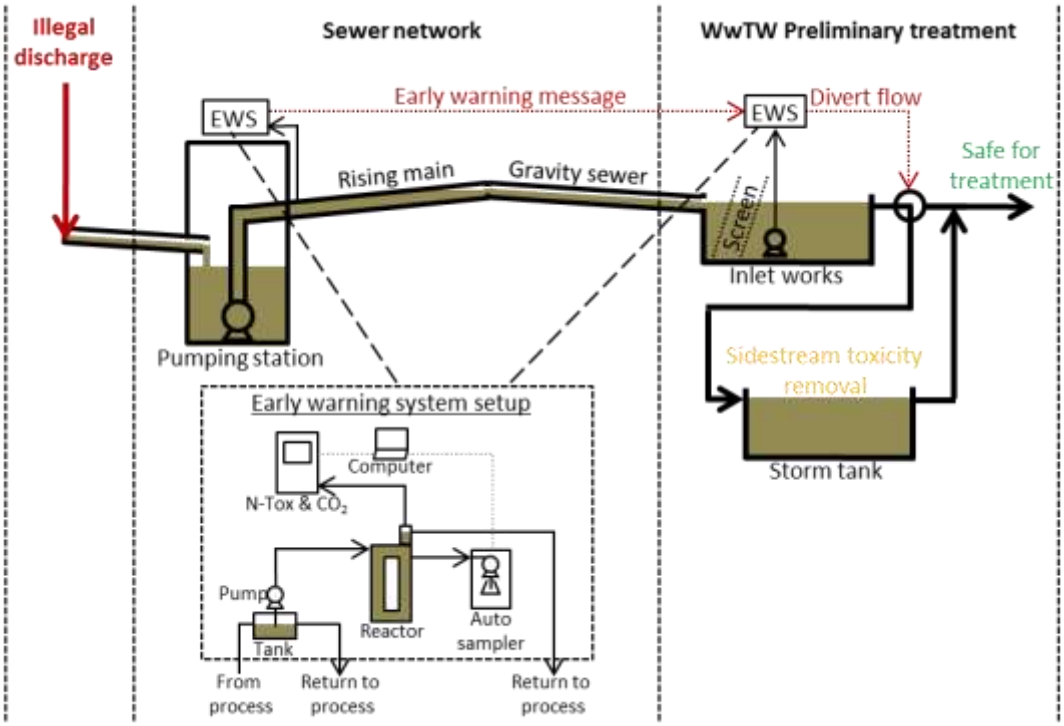


Figure 2 Early warning system (EWS) implementation setup in a full-scale sewer and wastewater treatment process

The aforementioned represents the basic EWS installation, to allow detection of acute toxicity. By expanding on this system and utilising a Nitritox DTA monitor at the inlet works, the nitrification inhibition percentage the toxic wastewater is likely to exhibit on the secondary treatment process can be measured. This would allow better rationalisation of the response and a more robust EWS. The system would also broaden the application of an EWS for detection of chronic toxicity as well as acute.

There is also potential to just monitor CO_2 emissions as the basis of the in-sewer CFBBR monitoring system, but still maintain the same toxicant detection spectrum as a system monitoring N_2O. The 13 % reduction in whole life cost represented by employing this setup would negate the additional costs associated with a Nitritox DTA monitor at the inlet works.

Overall, it was demonstrated in this study that a CFBBR biofilm with specific nitrification rates of ~0.4 g-NH_4^+-N.m^{-2}.d^{-1} can be utilised as an in-sewer EWS device. The system can effectively respond to concentrations of heavy metals known to be inhibitory to the secondary treatment process at the WwTW and proven as a viable device for catchments of >200,000 PE receiving industrial wastewater.

Keywords: Sewer, Biofilm, Toxicity, Early Warning System, Nitrification

CHAPTER 1. INTRODUCTION

Water utilities have a history of accepting discharges from various industrial operations (traders) for treatment at the wastewater treatment works (WwTW) (Farré and Barceló, 2003). With this, comes the inherent risk of undesirable events (e.g. a toxic plug) at the WwTW and accepting watercourse / ecosystem (Stasinakis et al., 2003; Xiao et al., 2015).

1.1 Framework of trader discharges

Under the Water Industry Act 1991 (The Crown, 2001), traders are required to obtain a consent to discharge into a sewer from the sewerage undertaker whereby they must declare:

- the nature and composition of the effluent
- the maximum proposed volume to be discharged
- the maximum proposed flow rate of the discharge

The sewerage undertaker has the power to impose conditions on the consent with respect to the nature and composition of the trade effluent. They can stipulate the maximum daily volume to be discharged and specify time slots of the day the trader can discharge. All these are taken into consideration when a consent is granted, and the trader is made fully aware of the consent conditions (The Crown, 2001).

If a trade effluent is discharged without a consent in place or the consent is breached, the sewerage undertaker has the right to prosecute the trader. If convicted, the trader could be liable to pay fines and the sewerage undertaker has the power to review the consent. For example, they may see fit to tighten the consent or restrict the trader to discharge at times of the day the discharge can be diluted (The Crown, 2001).

1.2 Historic toxic events

There have been a number of pollution incidents related to illegal discharges to sewer networks by traders. In the UK, Todmorden WwTW (Yorkshire Water) and Strongford WwTW (Severn Trent Water) have both experienced illegal discharges of trade effluent containing cyanide into their sewers. Both resulted in treatment failure at the WwTW, and subsequently led to partially treated sewage and cyanide in the final effluent. In

Todmorden, a 30 kg discharge of cyanide resulted in treatment failure, and a large scale fish kill in the river Calder (edie, 1999). The Strongford cyanide event caused complete treatment failure of the activated sludge plant (ASP), leading to a total fish kill along a 700 metre stretch of the River Trent, with traces of the toxic plug detected up to 30 miles away from the WwTW discharge point (Utility Week, 2009). In the United States, the major White River fish kill in December 1999 resulted in the death of hundreds of thousands of fish along a 50-mile stretch of the river, as a result of toxic trade effluent. Ammonium (NH_4^+-N), thiram, amines and an insecticide were detected in the WwTW's final effluent (edie, 2000).

Pollution events such as these, in addition to being detrimental to the environment, can result in major fines to the parties responsible, be it the water utility or a third party trader, and can severely damage public perception of all parties involved. Had the WwTW had some warning of the impending toxic events, they could have managed the situation and potentially mitigated a river pollution incident.

1.3 Providing an early warning

At present, random samples of industrial discharges are taken by wastewater companies in a policing manor, based on an agreed frequency (i.e. not all discharges are monitored). Due to temporal variability of effluent toxicity, a sample that is negative for toxicity does not necessarily mean the next sample will exhibit the same level of toxicity (USEPA, 2002). Toxic events are often experienced for short time periods (Kroiss et al., 1992), suggesting a high frequency of samples and long lead time is required to detect a toxic event. Hence, relying on effluent samples as the sole method for detecting toxicity is not a reliable approach suggesting an online monitoring system would be more appropriate.

1.4 In-sewer online early warning system

Online monitoring of crude sewage is rare but toxicity monitors are available that can be placed at the biological treatment stage of the WwTW. However, detection at this stage allows only a small mitigation time frame to protect the treatment process (if a toxic plug is detected) and continuously monitoring the discharge at every trader site is generally un-viable from a financial standpoint (Love and Bott, 2000). Hence, an in-sewer online early warning system (EWS), monitoring wastewater toxicity, could be the

missing link in protection of the WwTW and the subsequent accepting ecosystem from undesirable events (Black et al., 2014).

Sewers are hostile environments for sensor placement (Pedersen and Petersen, 1996). High concentrations of suspended solids, large particle sizes, suspended ragging and fats oils and greases (FOG) make pumping the low flows required for monitoring equipment difficult and can result in sensor fouling (Love and Bott, 2000). Filtration of a sample ahead of a sensor would minimise fouling issues on the sensor itself, however it would require frequent automated backwashing. As such, in-sewer toxicity monitoring equipment should employ a non-invasive sampling technique.

An in-sewer EWS would ideally detect a wide range of toxic substances ahead of the biological treatment stage of a WwTW (Black et al., 2014). Biological EWSs are the preferred monitoring option, as they can raise an alarm based on the impact a particular wastewater stream can have on the receiving biological treatment stage of the WwTW (Black et al., 2014). Toxicity monitors exist that detect an inhibition of either heterotrophic (i.e., organic carbon oxidisers) or autotrophic (i.e., inorganic compound oxidisers) biological activity.

1.5 Monitoring nitrifying bacteria stress responses

Autotrophic nitrifiers are one of the most vulnerable bacteria employed in biological wastewater treatment systems (Love and Bott, 2000). At WwTWs with final effluent ammonia discharge consents, secondary treatment processes are designed to favour nitrifier growth by limiting organic loading rates and increasing the age of the biomass. This typically leads to larger process units in comparison to units designed solely for carbonaceous removal of the equivalent crude wastewater load (Tchobanoglous et al., 2014a).

Nitrifiers are particularly sensitive to toxicants, with inhibition effects ranging from a reduction in growth rate or a reduction in specific nitrification rate (Burgess, Stuetz, et al., 2002). Hence, if sufficient inhibition to nitrifiers occurs, there is a risk of failing the final effluent ammonia consent. It is possible to measure the level of inhibition non-invasively by monitoring gaseous nitrous oxide (N_2O) emissions (Burgess, Stuetz, et al., 2002; Butler et al., 2009; Kim et al., 2010), and this could be employed as the basis of an online toxicity monitoring system.

However, the relative abundance of nitrifiers in cultures sustained with crude or settled wastewater (i.e., upstream of the biological treatment stage of a WwTW) is likely to be low (Baban and Talinli, 2009; Jiang et al., 2009). This brings the need for an environment designed to allow nitrifiers to be established. As the response time needs to be quick to capture toxic plugs as early as possible, hydraulic retention time (HRT) in this reactor needs to be low (typically < 30 minutes), bringing the risk of sludge washout in suspended growth systems. A biofilm system is therefore more favourable for the high load expected in the sewer network.

1.6 Biofilm based early warning system

Biofilms are typically composed of a complex mixture of microorganisms embedded in a layer of extracellular polymeric substances (EPS) or "slime" (Karunakaran et al., 2011). Depending on environmental conditions, the communities can be aerobic, facultative and / or anaerobic organisms, with a mixture of this generally assumed to be the case in most wastewater environments. Whilst a biological EWS would ideally have a predominantly nitrifying community, the presence of heterotrophs can be advantageous. Firstly, heterotrophs aid attachment of nitrifiers to a surface, due to their comparably high excretion of EPS. Nitrifiers produce EPS in low quantities, and have been reported to be poor biofilm formers on their own as a result (Bassin et al., 2012). Secondly, heterotrophs can act as a protective layer, retaining nitrifiers within the biofilm (Bassin et al., 2012). Thirdly, whilst less sensitive than nitrifiers, heterotrophic bacteria can also elicit a response to some toxic shocks and could potentially act as a fail-safe for the detection of a toxic event where nitrifiers are completely inhibited and allow utilisation of the EWS in catchments without a final effluent ammonia consent. The challenge is in providing the conditions for nitrifiers to co-exist with heterotrophic bacteria under the high organic loading rates in crude sewage, low HRT required for a quick response and potentially low dissolved oxygen (DO) concentrations.

The negative effect of a high organic loading rate (>200 g-COD.m^{-2}.d^{-1}) can be overcome through spatial separation of heterotrophic and autotrophic activity, coupled with short HRT. Such an environment can be created using a circulating floating bed biofilm reactor (CFBBR) (Cui et al., 2008; Eldyasti et al., 2011; Lazarova and Manem, 1996; Li et al., 2012; Nogueira et al., 2002). A CFBBR is a three phase vertically orientated reactor consisting of an aerated riser shaft and non-aerated down comer shaft

connected at the top and bottom, with biofilm supported on floating plastic carrier elements. Anaerobic heterotrophic activity is favoured at the point of injection of the wastewater, in the down-comer. The anoxic conditions enable chemical oxygen demand (COD) in the feed to be reduced and when exposed to aeration in the riser, the metabolic activity of nitrifiers and / or aerobic heterotrophs (AH) is favoured. A homogenous three phase circulating flow through a differential pressure gradient across both shafts is thus maintained (Lazarova et al., 1998). For secondary wastewater treatment applications, an HRT of 50 minutes has been shown to limit accumulation of new bacteria / sludge, ensuring longevity of nitrifiers' activity in a pre-grown mixed population biofilm (Nogueira et al., 2002). This could be adapted for a reactor upstream of the biological treatment stage of a WwTW.

1.7 Gaseous emissions as a toxicity response

The biofilm response to toxicity occurs within a multistep process of nitrogen and carbon removal from wastewater (Figure 1.1), the interruption of which differs depending on the toxicant applied. There are two main routes to detection of toxicity based on gas emissions: direct and indirect. The latter, results from the initial inhibtion of aerobic respiration, which in turn results in the transformation of an otherwise anoxic environent to a microaerobic one. Under hypoxic conditions, the final step of denitrification is inhibted, which results in the accumulation and emission of N_2O and carbon dioxide (CO_2) gas. The route to emissions begins with an accumulation of nitrite (NO_2^-) and a positive concentration gradient of NO_2^- over hydroxylamine (NH_2OH). As NH_2OH is highly toxic to ammonium oxidising bacteria (AOB) and methanotrophs they quickly act to reduce it either through step 1a back to ammonium (NH_4^+) or to nitric oxide (NO) in step 2a (Figure 1.1) terminating at N_2O in step 6a (Desloover et al., 2012; Stein and Klotz, 2011). Once a negative concentration gradient between NO_2^- and NH_2OH is achieved step 2 can resume (Figure 1.1), where the AOB and methanotrophs then reduce NO_2^- to N_2O in steps 5a and 6a. In addition, the enzyme nitrous oxide reductase (NOS), required to complete denitrification by reducing N_2O to N_2 in step 7 (Figure 1.1), is inhibited by high oxygen concentrations (Short et al., 2014). Due to inhibition of the aerobic micro-organisms (autotrophs and AH) DO concentration increases, resulting in a hypoxic environment that is sub-optimal for denitrification (Short et al., 2014). The end result is incomplete denitrification, accumulation of N_2O

and a gaseous emission (Debruyn et al., 1994; Desloover et al., 2012; Short et al., 2014).

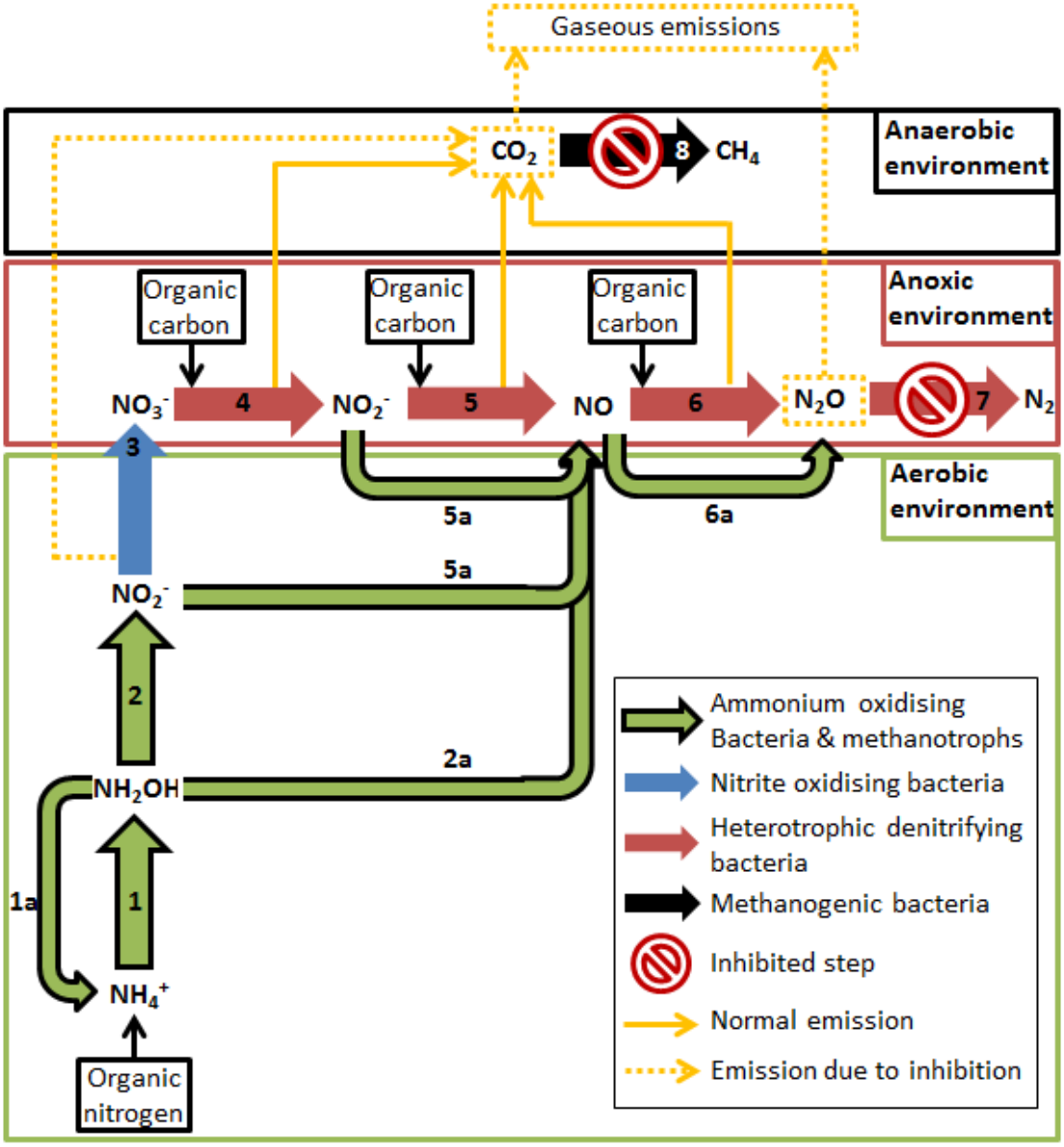


Figure 1.1 Transformation pathways for nitrogen and carbon in a mixed culture receiving domestic wastewater, including the main microbial group's responses to a heavy metals shock. Step 1; Ammonium oxidation by ammonium oxidising bacteria (AOB) with ammonium mono-oxygenase (AMO) (Desloover et al., 2012; Stein and Klotz, 2011). Steps 1a, 2 and 2a; hydroxylamine reduction by AOB and methanotrophs with hydroxylamine oxyreductase (HAO) (Desloover et al., 2012; Stein and Klotz, 2011). Step 3; nitrite oxidation by nitrite oxidising bacteria (NOB) with nitrite oxidoreductase (NXR) inhibited by heavy metals. Step 4; Nitrate reduction by heterotrophic denitrifying bacteria (HDN) with membrane bound nitrate reductase (NAR), periplasmatic nitrate reductase (NAP) (Desloover et al., 2012; Stein and Klotz, 2011) and an organic carbon source (OC). Steps 5 and 5a; Nitrite reduction by AOB, methanotrophs and HDN with nitrite reductase (Nir) (Desloover et al., 2012; Short et al., 2014; Stein and Klotz, 2011). Steps 6 and 6a; nitric oxide reduction by AOB, methanotrophs and HDN with nitric oxide reductase (Desloover et al., 2012; Short et al., 2014; Stein and Klotz, 2011). Step7; nitrous oxide reduction by HDN with nitrous oxide reductase (NOS) inhibited by heavy metals. Step 8; methanogenesis by methanogenic bacteria inhibited by heavy metals results in excess CO_2 accumulation from steps 4, 5 and 6 in addition to a reduction in CO_2 fixation in step 3 due to inhibition.

Examples of toxicants with an inhibitory action like this are copper (II) and chromium (VI). In the case of copper (II), both the NH_4^+ and NO_2^- oxidation electron transfer chains are abundant with copper containing enzymes (Painter, 1970). However, the requirement of copper for the primary nitrification enzyme ammonium mono-oxygenase (AMO), translates to a lower inhibitory effect on NH_4^+ oxidation in step 1 (Figure 1.1) over NO_2^- oxidation in step 2 (Barber and Stuckey, 2000), resulting in NO_2^- accumulation. The heterotrophic denitrifying bacteria (HDN) and AH have been shown to be more susceptible to the copper (II) inhibitory effect than the nitrifying community (Ochoa-Herrera et al., 2011). Conversely, chromium (VI) presents a much lower inhibitory effect than copper (II), with both HDN and nitrifying communities experiencing similar sensitivities to chromium (VI) (Madoni et al., 1999). As such, the most likely cause of N_2O emission during chromium (VI) shock loads is again the hypoxic environment as a result of reduced respiration of aerobic micro-organisms.

Direct detection of toxicity through gaseous emissions results from a transient accumulation of NH_4^+ which is inhibitory to nitrite oxidising bacteria (NOB) (Chandran and Smets, 2000). The HDN continue to reduce NO_3^- to N_2O in steps 4, 5 and 6, however, as with copper (II) and chromium (VI), the increase in oxygen concentration again permits N_2O to accumulate and a gaseous emission to evolve (Figure 1.1). At the same time, CO_2 fixation rate by NOB in step 3 (Oguz et al., 2006; Tchobanoglous et al., 2014b) and conversion rate to methane (CH_4) in step 8 (Capone et al., 1983; Sanchez et al., 1996; Tchobanoglous et al., 2014b) reduces due to inhibition, resulting in accumulation and gaseous emission (Figure 1.1). Likewise, under anaerobic conditions, likely to occur in the deep layers of wastewater biofilms (Bassin et al., 2012; Tchobanoglous et al., 2014a), inhibition of methanogenic organisms can result in CO_2 accumulation and emission (Figure 1.1). Hence, CO_2 can also give an indication of microbial activity in addition to N_2O. Nickel (II) is an example of a toxicant with this inhibitory action. It has been demonstrated that the nitrification process is significantly more sensitive than heterotrophic processes, with NH_4^+ oxidation through step 1 (Figure 1.1) generally more sensitive than NO_2^- oxidation in step 2 (Hu et al., 2004).

In some cases, no gaseous response should be expected. This is likely to be the case with ATU which is a known inhibitor of ammonium oxidation in step 1 (Figure 1.1) but

presents no inhibitory effect to HDN or AH (Butler et al., 2009). In this case a hypoxic environment is not likely to be created as the only aerobic organisms affected would be the autotrophs. As such, no inhibition to NOS would be experienced and there would be no route permitting N_2O accumulation and emission (Figure 1.1). In addition, CO_2 would continue to be converted to CH_4 in step 8 (Figure 1.1), as ATU is not known to be toxic to methanogens (Capone et al., 1983; Sanchez et al., 1996). Despite this, N_2O emissions have been reported to increase in the presence of ATU accompanied by NH_4^+ accumulation (Burgess, Stuetz, et al., 2002; Butler et al., 2009). Whether the origin of this is partial interruption of NH_4^+ oxidation in step 1 (i.e., nitrification continues but under higher NH_4^+ concentration conditions thus under stress) or heterotrophic denitrification under aerobic conditions (Krul and Veeningen, 1977) is unclear, but demonstrates the potential of N_2O emissions generation as part of the biological response to ATU. However, this is unlikely to be detectable based on ATU's inhibitory action.

Building on the interepretation of these cycles and interactions and integrating the water utility's need for a better approach to toxic events, this study aimed to characterise the response of biofilms to toxicity. From this, the suitability of monitoring the gaseous emission of biofilm toxicity as an in-sewer EWS was assessed, by studying the difference of toxicant inhibitory effect on biofilm and suspended growth systems. The practicalities of an EWS were also appraised in terms of risk, benefits and whole life costs.

1.8 Characterising toxic events

The nature of toxic events is widely varied and may be transient or enduring (Love and Bott, 2000). The relative impact of transient events is acute and characterised by either temporary or catastrophic treatment failure, depending on the concentration of toxicant and the type of toxicant (Quinn et al., 2008; Tyagi et al., 2012). The impact to the receiving watercourse will also be sudden and severe (e.g. fish kills), however lasting environmental damage is unlikely (USEPA, 2000). Such events are rare and therefore the likelihood of initial occurrence or repeat incidents is low. Nevertheless, the Strongford cyanide incident that occurred in 2009 in Staffordshire, UK illustrates how

the severity of such events can be high. Therefore, the overall risk of these incidents can be extremely severe.

Acute events will likely result in detectable N_2O (Burgess, Stuetz, et al., 2002; Butler et al., 2009; Kim et al., 2010) and CO_2 (Capone et al., 1983; Oguz et al., 2006; Sanchez et al., 1996; Tchobanoglous et al., 2014b) peaks, with respect to the emissions pathways described previously (Figure 1.1). Hence, this work focuses on development of an EWS for detection of acute nitrification inhibitors based on nitrifiers gaseous stress responses.

Sub-lethal concentrations of toxicant can also induce microbial stress but might not have a significantly adverse impact on microbial activity (Giller et al., 1998; Love and Bott, 2000; Quinn et al., 2008; USEPA, 2000). However, long-term exposure to these concentrations may lead to a gradual drop in treatment performance and eventual chronic treatment failure. It is also far more likely that these events will lead to lasting environmental damage, e.g. loss in biodiversity, ecological population numbers and plant life (USEPA, 2002).

Due to the sub-lethal nature of the chronic events (Giller et al., 1998; Love and Bott, 2000; Quinn et al., 2008; USEPA, 2000), it is potentially difficult to provide an early warning upstream of the treatment process through microbial stress responses such as N_2O and CO_2 emissions, and would require equipment capable of measuring either the concentration of specific chemicals or the percentage inhibition. Respirometric biosensors are potentially suited to this application and this option has been explored in this study to expand the application of the EWS to chronic events.

1.9 In-sewer implementation of an EWS

Sewers may operate under gravity or under pressure (Langeveld et al., 2002). The key difference between gravity and pressure sewer mains is the variation in flowrate. The peak and minimum flow rates ($l.s^{-1}$) in a gravity sewer are dictated by the temporal volume of sewage draining into it . The peak flow rate may differ day to day depending on the behaviour of discharge into it (Langeveld et al., 2002). The minimum flow in a gravity sewer is typically taken to be the level of infiltration into it. The depth of sewage within the pipe is related to temporal discharge volume (Severn Trent Water, 2009a). Hence, due to the varying depth, the EWS sample supply tube must sit within the sewer

pipe, posing a risk of catching rags and fat deposits. This could result in a sewer blockage and a subsequent sewer flooding / pollution incident.

By contrast, pressure mains operate completely full, on a fill and draw operational regime of a pumping well fed by a gravity sewer. The total daily volume passing through the pressure main is the same as the gravity sewer feeding the well, however unlike the gravity sewer the peak flow rate is a constant dictated by the pumping duty and the minimum flow rate is 0 $l.s^{-1}$ (Severn Trent Water, 2010).

Pressure mains are far better suited to the CFBBR based EWS. The CFBBR feed could be taken from a tapping point on the pressure main, using the existing pumping head to deliver sewage to the EWS, posing no risk of sewer blockage.

1.10 PR14 justification for an in-sewer EWS

As part of price review 14 (PR14), the UK's wastewater treatment companies have made specific commitments to maintain compliance with WwTWs discharge licenses (zero treatment failures) and improve environmental quality (Table 1.1). To reflect the high cost of achieving these commitments and the benefits customers will forgo if the commitments are not delivered the relative penalties and rewards set by OFWAT as outcome delivery incentives (ODIs) in its final determination are high (Table 1.1).

Table 1.1 Wastewater treatment company commitments to treatment compliance and agreed OFWAT outcome delivery incentives (OFWAT, 2015a, 2015b, 2015c, 2015d, 2015e, 2015f, 2015g, 2015h, 2015i). Penalty and reward rates are per unit, i.e. per discharge point.

Company	Commitment	Outcome delivery incentives	
		Penalty rate	Reward rate
Anglian Water	S-C1: Percentage of bathing waters attaining excellent status	£373,000	£373,000
	S-S3: Pollution incidents	£28,500	£28,500
	S-C4: Environmental compliance	£620,000	-
Northumbrian Water	S-C1: Sewage treatment works discharge compliance	£2,228,625 initial lump sum followed by a second £2,228,625 lump sum	-
	S-C2: Pollution incidents (category 3)	£2,228,625 initial lump sum followed by a second £2,228,625 lump sum	£16,000
	S-C3: Bathing water compliance	£113,000	-
Severn Trent Water	S-C1: Improvements in river water quality against WFD criteria	£150,000	£150,000
	S-C2: The number of category 3 pollution incidents	£53,900	£53,900

	S-C7: Overall treatment performance	£2,400,000	£2,400,000
South West Water	Wastewater treatment numeric compliance	£296,000	-
	Wastewater descriptive works permit compliance	£250,000	-
	Pollution incidents (category 1&2)	£346,000	-
	Pollution incidents (category 3&4)	1st penalty £10,300 2nd penalty £19,300	-
	Bathing water quality	£103,000	£249,000
	Category 1 & 2 pollution incidents	£346,000	-
Southern Water	Wastewater treatment works numeric compliance	£1,661,000	-
	Bathing waters with excellent water quality	£3,640,000	£246,750
Thames Water	SC2: Total category 1-3 pollution incidents from sewage related premises	£130,000	£130,000
	SC3: Sewage treatment works discharge compliance	£3,845,000	-
United Utilities	S-C1: Contribution to bathing waters improved	£10,000,000	-
	S-D1: Protecting reviews from deterioration due to population growth	£58,000	-
	S-D3: Contribution to rivers improved	£111,000	£28,000
	S-D4a: Wastewater serious (category 1 and 2) pollution incidents	£420,000	-
	S-D4b: Wastewater category 3 pollution incidents	£282,000	£149,000
Wessex Water	S-B1: The EA's environmental performance assessment	£5,900,000	£190,000
	S-B3: River water quality improved	£1,674,000	£1,290,000
Yorkshire Water	SA3: Pollution	£185,133	£185,133
	SB2: Wastewater quality stability and reliability factor	Up to 10% TOTEX	-
	SB4: Length of river improved	£146,238	£76,696

As such, an EWS has significant potential in assisting UK wastewater treatment companies to achieve their treatment performance commitments, as policing treatment failures due to toxic discharges into the sewer will need to be stringent. The scale of the ODIs could make it viable to employ an EWS in all catchment sizes and receiving watercourse types. Close attention must be paid in rationalising the response at different locations when employing a CFBBR based EWS.

The mixed culture of the CFBBR biofilm consists of nitrifiers and carbonaceous removal bacteria. It is well suited to inland catchments (discharging to rivers) receiving industrial wastewater, presenting a risk of toxicity at the WwTW. Treated effluent discharges to inland rivers typically have an ammonia and biochemical oxygen demand (BOD) consent in place (United Utilities Plc, 2008) so a CFBBR based EWS monitoring gaseous emissions will give a response with respect to nitrification and carbonaceous removal inhibition.

By contrast, ammonia consents are not typically applied to coastal discharges thus F/M ratios of secondary treatment processes are configured high to favour carbonaceous removal (United Utilities Plc, 2008). An ASP sized for a coastal catchment will typically be smaller when compared to an inland catchment of the same PE as a result of the high F/M (United Utilities Plc, 2008). As such, responses due to nitrification inhibition in the CFBBR biofilm should be taken into account when rationalising an EWS toxicity response in a coastal catchment.

For the purpose of testing the principal of a CFBBR based EWS monitoring N_2O and CO_2 emissions as a stress response, sewage spiked with substances expected in industrial effluents has been tested. As such, the suitability of the EWS for sites receiving industrial effluents has been tested. For smaller catchments with little or no industrial discharges, the risk of treatment failure due to toxicity is low. However, these catchments can be subjected to illegal fly tipping into sewer manholes that have the potential to result in inhibition of treatment at the WwTW. In light of the high ODIs for environmental compliance an EWS could be viable even when there is a low risk of toxicity.

CHAPTER 2. RESEARCH AIMS AND OBJECTIVES

The overall aim of the project is to characterise the response of biofilms to toxicity and establish the suitability for application in an in-sewer early warning system.

To fulfil this aim, key gaps in knowledge were identified and used to form the objectives of this research. Overall five objectives were identified:

1. Identify a commercially available toxicity monitor that could be adapted for use in a biological EWS.
2. Establish the minimum requirements for sewer deployment of an EWS device.
3. Design a suitable environment to sustain a biofilm at high loading rates.
4. Characterise the response of biofilms to toxicity and compare it against the response of suspended growth cultures.
5. Develop an EWS based on the adapted monitor and profile biofilm responses to toxicity.

This thesis is structured to demonstrate how each of these objectives was met. A review of commercially available online wastewater toxicity monitors (CHAPTER 3) was carried out to address objective 1 and provide the basis for undertaking the following objectives. Objectives 2, 3, and 4 were addressed by a step-wise approach including an experimental and design work phase (CHAPTER 4). The implementation of the research outputs from Chapter 4 has then been carried out to address Objective 5 (CHAPTER 5). The thesis then provides a final discussion and a holistic and critical outlook of EWSs (CHAPTER 6) and recommendations for future work (CHAPTER 7).

CHAPTER 3. REVIEW OF ONLINE MONITORING DEVICES USED IN WASTEWATER TREATMENT

3.1 Introduction

Biological treatment of wastewater is the most cost-efficient method for treating sewage. However, by its very nature, it relies on a healthy community of microorganisms to deliver the level of treatment required, leaving it vulnerable to failures in the treatment process when receiving toxic influent loads (Xiao et al., 2015). In spite of this risk, there is limited implementation of toxicity monitoring at WwTWs. Moreover, because of the difficulties associated with sensor fouling, sample pipe blockages, and accessibility to name a few, there is no reported in-sewer monitoring of toxicity. This work proposes a step change in on-line wastewater toxicity monitoring, by critically reviewing commercially available monitoring technologies that could be potentially implemented or adapted for use in the sewer network.

This chapter reviews the online monitoring devices used in wastewater treatment and it is divided into three sections. The first section provides a review of the literature for pollutant transformations in the sewer and defines the criteria for deployment of an EWS to the sewer (in terms of practical considerations and toxic event risk reduction). The second section describes the available monitoring technologies, and their suitability for in-sewer uses. The reduction in risk of a chronic and acute toxic event as a result of installing an EWS is scored for each monitor type. In the third section, monitoring technologies have been broken down into discrete categories, scored against a set of weighted criteria and plotted against the retail cost in order to assess their suitability and cost effectiveness. Additionally, the performance of the monitoring devices with respect to the substance range they respond to has been analysed to further assess the cost effectiveness and feasibility for full-scale implementation in the sewer network. From this, the most suitable technology for an in-sewer EWS is recommended and an insight is provided into the strengths and weaknesses. The chosen technology from this section forms the basis of the in-depth EWS studies and trials discussed in the Chapters 4 and 5.

3.2 The sewer environment

Municipal combined sewer systems (CSSs) collect and convey wastewater from domestic, industrial and commercial properties (as well as storm water runoff) to the WwTW for treatment (Ashley et al., 2000). As such, fluctuations in volume and composition of WwTW influent can be related back to conditions in the CSS (Langeveld et al., 2002). Understanding the biological, chemical and physical transformation processes within the sewer environment is critical for determining the suitability of an online toxicity monitor.

3.2.1 Pollutant transformations, storage and release

Biofilm formation in sewers has been linked to organic matter availability and periods of low flows (Jiang et al., 2009). Structural and hydraulic discontinuities in CSSs promote sedimentation of organic and inorganic solids within sewers in dry weather flow (DWF) and under decelerating flows towards the end of a storm event (Banasiak et al., 2005). This residual organic substrate can ultimately encourage the natural development of a biofilm along the walls of the pipe and at the water-sediment interface (Ahyerre et al., 2001; Chen et al., 2003).

Sewer biofilms are widely regarded as a problem in relation to odour and corrosion (Tanner, 2008), however, they play an active role in pollutant transformation (Jiang et al., 2009) and storage (Skipworth et al., 2000). Soluble and particulate heavy metals can experience changes in their physiochemical structure during conveyance in the sewer. Precipitation of sulphide and phosphate minerals or metal alloy sulphurisation can occur under anaerobic conditions (Houhou et al., 2009). The result is a residual concentration of heavy metals within sewer sediments under DWF.

The formation timeline of the sewer biofilm has been reported to commence with a 10 day period of exclusive heterotrophic growth (Jiang et al., 2009). Sulphate reducing bacteria (SRB) emerge in the deep, anoxic layers at approximately the 30-day mark. As the biofilm thickness increases, the SRB grow rapidly, occupying a larger portion of the biofilm. Maturity is reached after 90 days, with a stable thickness and constituency. At this point, SRB occupy the largest percentage of the biofilm, however, their numbers within the deep layers reduces due to limited substrate penetration. It has been reported

that nitrifiers almost disappear once maturity is met, failing to compete with other microbes in the biofilm (Baban and Talinli, 2009; Jiang et al., 2009). It is, however, important to consider the effects of time varying flow on the maturity of the biofilm.

The rapid increase in suspended sediment and pollutants at the beginning of a storm event is defined as the "first foul flush" (Skipworth et al., 2000). The wide range of organic and inorganic suspended solid sizes and configurations, coupled with the time varying flows result in disturbance of the sewer biofilm at the water sediment interface (Banasiak et al., 2005). Mobilisation of the sewer sediments can be responsible for up to 50 % of the total pollutant concentration in the wastewater during a first foul flush event (Gromaire et al., 2001). Therefore, the level of biofilm maturity and thickness is dictated by the frequency of rainfall events.

3.2.2 On-line sewer monitoring: practical considerations

Toxicity monitor selection is typically based on their sensitivity to known toxicants, response time and reliability of the response (Love and Bott, 2000). For an in-sewer deployment, additional criteria for the evaluation of monitors include practical considerations, namely: sampling method, housing protection rating, maintenance frequency, response time, connectivity, and power source. This review has assessed commercially-available monitors based on these criteria, which are further described as follows:

a. Sampling method

A sewer may be considered a hostile environment for sensor placement (Pedersen and Petersen, 1996). High concentrations of suspended solids, large particle sizes, suspended ragging and FOG make pumping difficult and can result in sensor fouling (Love and Bott, 2000). The likelihood of sensor fouling is directly proportional to the invasive nature of a monitoring technique; e.g., gas phase monitoring is less prone to fouling when compared against liquid phase monitoring. Whilst filtration of a sample ahead of a sensor would minimise fouling issues on the sensor itself, it would require frequent automated backwashing. As such, higher scores were administered to monitors that were non-invasive, followed by those with built-in filtration and cleaning, followed by unprotected invasive sensors.

b. *Housing protection rating*

To maximise the potential application of monitor deployment in the sewer, it should be amenable to being placed in a remote location, possibly un-sheltered above ground. To allow continuous un-interrupted operation, the effects of weathering and vandalism on the inner workings of the monitor need to be minimised. To achieve this, the outer housing for the monitoring equipment needs to have a high level of protection. Monitors were thus scored highly when the specifications stated a high ingress protection (IP) rating.

c. *Maintenance frequency*

In a remote location, the monitor may be expected to operate for long periods of time, un-aided. The monitor may also form one part of a much wider network of equipment. To meet this expectation and brief, maintenance requirements and frequency need to be minimal. In addition, the level of skill required to operate the equipment should be minimal, to encourage widespread use. Monitors were thus scored highly when they required low quantities of consumable and minimal parts replacements.

d. *Response time*

The probability of obtaining a discrete sample at the exact moment of a toxic event may be low. Hence, the presence of monitoring equipment capable of identifying a sudden change in wastewater composition could prove valuable (Bourgeois and Stuetz, 2002). On-line monitoring equipment should return a high resolution data-set in order to build an accurate response profile. The monitor's reading time should be fast and ideally in real-time, allowing the maximum time window for mitigation of an un-desirable event, and the highest resolution of output data for construction of time profiles. Monitors were thus scored highly when they had a short response time and had short periods between data collection points (i.e. high data resolution).

e. *Connectivity*

The monitor should have a universal connectivity method (such as USB or LAN) for integration into the industry standard supervisory control and data acquisition system

(SCADA). This allows rapid response by operational teams and real-time correlation with WwTW performance. Monitors were thus scored highly when they had a modem / internet connection fitted (preferably mobile internet to allow remote installations), they had a bespoke data logging setup easily accessible on-line or when data could be easily downloaded via USB.

f. ***Sensitivity***

It is essential that the monitor can detect a small response range, to characterise a toxic event. The "limit of detection" and resolution (in ppm) have been used to assess the sensitivity of a device.

g. ***Power source***

The power requirement is also an important factor to consider. In order to deploy to remote locations, a long life battery pack may be preferential. Otherwise, single phase 110-230 V should be the standard, to aid ease of installation (Love and Bott, 2000). Monitors were thus scored highly when they had low power demands and could be powered by battery.

3.2.3 Toxic event risk assessment and mitigation with an EWS

Acute events are transient in nature, characterised by high toxicant concentration and sudden severe impact to the treatment process / environment (Table 3.1). Chronic events are enduring in nature, typically characterised by sub-lethal concentrations, gradual reduction in treatment performance and long lasting environmental impact (Table 3.1). In the absence of an EWS, the risk associated with acute and chronic toxic events is high and medium / high respectively (Table 3.1).

Table 3.1 Risk associated with toxic events in the absence of an EWS.

Events		Acute toxic events	Chronic toxic events
Transient in nature		✓	✗
Enduring in nature		✗	✓
High toxicant concentration		✓	✗
Sub lethal toxicant concentrations		✗	✓
Sudden severe impact to watercourse		✓	✗
Long lasting environmental effect		✗	✓
Risk Scoring*	**Likelihood of event (1-5)**	4	4
	Severity of event (1-5)	5	4
	Overall toxic event risk score	**20**	**16**

*refer to Figure 3.1 for risk scoring

Hence, in addition to the scoring criteria in section 3.2.2, monitoring equipment was also appraised by the reduction in risk of a toxic event associated with installation of the device. A score equal to the reduction in risk was added to the overall score for the device. An EWS will not adjust the likelihood of a toxic event occurring, however it will adjust the severity of impact and hence the overall risk. With an overall risk of 20 (Table 3.1), an EWS is highly justified for risk reduction of an acute toxic event (Figure 3.1). Similarly, an overall risk of 16 for chronic toxic events (Table 3.1), also justifies installation of an EWS for risk reduction (Figure 3.1).

To detect acute toxicity and reduce the severity of an acute toxic event, the monitoring equipment needs to be capable of responding quickly to lethal concentrations of toxicant. For chronic toxicity detection, the monitoring equipment needs to be capable of detecting sub-lethal concentrations of toxicant.

Risk = Likelihood x Severity

Severity							
	Catastrophic	5	5	10	15	20	25
	Major	4	4	8	12	16	20
	Serious	3	3	6	9	12	15
	Minor	2	2	4	6	8	10
	Negligible	1	1	2	3	4	5
	Likelihood (Probability)		1 Extremely Unlikely	2 Very Unlikely	3 Unlikely	4 Likely	5 Very Likely

1 – 4: **Low risk** – Low driver for EWS installation

5 – 14: **Medium risk** – Risk controls require preventative measures. An EWS may be justified

15 – 19: **Medium / high risk** – Risk controls require preventative measures. An EWS may be justified

20 – 25: **High risk** – Risk controls require preventative measures. An EWS is justified

Definitions of Probability of (Likelihood)			Definitions of Severity		
Likelihood (Probability)		Definition	Consequences (Severity)		Definition
Very Likely	5	Certain to occur	Catastrophic	5	Severe environmental damage
Likely	4	Likely to occur	Major	4	Significant environmental damage
Likely	3	Possible to occur	Serious	3	Serious environmental damage.
Very Unlikely	2	Unlikely to occur	Minor	2	Minor environmental damage.
Extremely Unlikely	1	Remote occurrence	Negligible	1	Negligible impact

Figure 3.1 Toxic event risk matrix.

3.3 Wastewater monitoring devices for water quality purposes

The available wastewater monitoring technologies were broken down into five key categories based on the nature of the detection, namely; biosensors, gas monitors, voltammetry, wet chemistry and solid state. The areas of interest in wastewater monitoring were quantified by the number of papers reviewed in relation to each monitoring category (Figure 3.2).

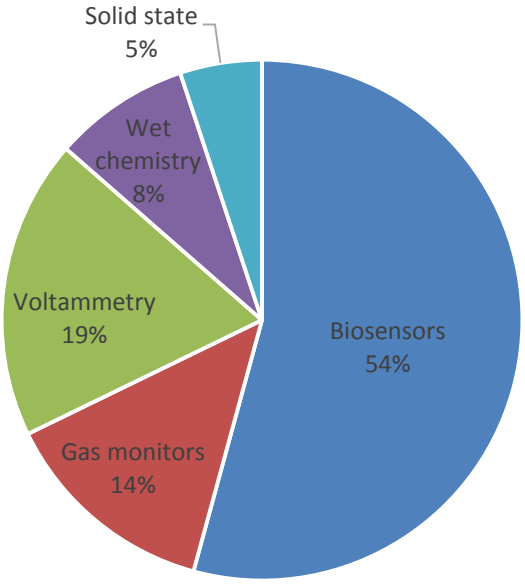


Figure 3.2 Distribution of monitoring types studied in published literature (n = 59).

Thirty five monitoring devices within these categories were evaluated according to the criteria described in section 3.2.2. A summary of the device specifications is presented in Table 3.2 and their detection principles and likely suitability for an EWS is discussed hereinafter.

Table 3.2 Monitoring device specification.

Manufacturer and device	Category					Intended location				Sampling method				Housing Protection						Response Time (Minutes)	Maintenance Frequency			Skill level		Connectivity					Power				Toxic event adjusted risk score		Sensitivity (ppm or stated)	Cost (thousands £GB)
	Biosensors	Gas monitors	Voltammetry	Wet chemistry	Solid state	In-Sewer	Inlet	ASP	Final Effluent	Direct	Filtered	Headspace	In-situ	IP54	IP55	IP65	IP66	IP67	IP68		Monthly	6 monthly	Annually	Low	Medium	USB	LAN	SD card	RS232	GPRS/GSM	100-230VAC	230VAC	12-18VDC	18-36VDC	Acute toxic events	Chronic toxic events		
Challenge ODM-100	■							■		■						■				1	■				■	■	■				■				16	12	0.01	-
Kelma RODTOX	■							■		■						■				30	■				■				■		■				8	12	0.1 %	21
Isco-STIP STIPTOX	■							■		■						■				3-15	■				■				■			■			8	12	0.1 %	33
Applitek Microtox CTM	■							■		■						■				30	■				■	■	■		■		■				8	12	0.1 %	37
LAR Nitritox	■						■	■	■	■						■				5	■				■	■	■		■		■				8	12	0.1 %	22
Applitek Ra-BOD	■										■				■					3-15	■				■	■	■					■			20	12	0.01	-
Isco-STIP Biox1010	■						■		■		■			■						3-15	■				■				■			■			20	12	5	-
Water innovate N-TOX		■						■				■				■				1		■		■		■	■	■			■				8	16	0.1	18
Gas Data Click!		■										■								1				■		■					■	■	■		8	16	0.1	-
App-Tek OdaLog RTx		■				■						■							■	1				■								■			12	16	0.1	5
Multi-sensor MS1100		■				■	■					■					■			1		■			■	■					■		■		8	12	$1x10^{-3}$	-
Cogent OVA series			■				■	■	■	■						■				30	■				■	■	■		■		■	■			8	12	$5x10^{-4}$	23
Applitek VPA			■							■					■					30	■				■	■	■				■				8	12	$5x10^{-4}$	-

Applitek TONI																				3-15															20	12	0.01	-
Applitek TOPHO																				3-15															20	12	0.01	-
Applitek NIPHO																				3-15															20	12	0.01	-
Applitek AppliCOD																				3-15															20	12	0.01	-
Applitek AppliTOC																				3-15															20	12	0.01	-
Applitek EPA																				15															20	12	0.01	20
Applitek Envirolyzer																				15															8	12	0.01	-
Envitech AmNiSys																				1															20	12	0.1	-
Isco-STIP PHOENIX-Thermcat																				3-5															20	12	4	-
Isco-STIP Spectron																				12															20	12	0.1	-
sco-EZ-TOCII																				<8															20	12	0.1	-
Isco-STIP PBS																				3-5															20	12	0.1	-
Hach-Lange LDO																				0															16	16	1x10^{-6}	1.7
Hach-Lange pHC																				0															16	16	0.01 pH	1.7
Insite-IG OptoDOSys:M																				0															16	16	0.01	-
GOsys:ISA																				1-10															20	12	0.1	-
TriOs PROPS																				1															20	12	0.1	-
sco-Stipscan																				1-10															20	12	0.1	-
TriOs Enviroflu-HC																				1															20	12	1x10^{-4}	-
MJK pHix-compact																				1															16	16	0.01 pH	-
MJK SolSys:XL																				1															20	12	1	-

3.3.1 Biosensors

a) Biosensors monitoring respiration inhibition

Respirometry monitors the oxygen uptake rate (OUR) of a biomass, to measure the metabolic rate of the bacteria. Most of this energy is used for their biosynthesis and growth, hence, the OUR is a good estimator of the combined biomass growth rate of heterotrophs and autotrophs (Vanrolleghem and Lee, 2003). Any drop in OUR as a result of a toxicant can be characterised as a percentage inhibition, allowing the monitor to respond to a broad range of substances. This ability is termed direct toxicity assessment (DTA) whereby the effects of a toxicant on a biological system are used to monitor wastewater toxicity.

Traditionally, respirometric devices monitor the respiration rate of activated sludge (AS) batch fed from the ASP of interest. This can limit the location of the monitor to either the vicinity of the ASP or, at most, within the same WwTW with the aid of pumping and piping to seed the system. A respirometric device typically consists of a measurement cell and a DO probe. The sample is pump fed at set intervals to the measurement cell. The OUR of the sample is calculated over a fixed period either through the differential in liquid phase DO concentration, or the partial pressure of oxygen in the headspace above the sample. An inhibition percentage is subsequently returned.

The RODTOX (Rapid Oxygen Demand and TOXicity tester) is a respirometric monitor with a 10 litre supply of suspended biomass, kept under similar conditions to the target ASP. Favourable conditions for biological activity are maintained through continuous aeration and a controlled temperature. The DO concentration and pH of the sludge are measured every 2 minutes, and the OUR is monitored on-line (Geenens and Thoeye, 1998; Kong et al., 1993). When the DO in the measurement vessel is at its baseline concentration, the endogenous phase has been reached. At this point, a pulse of sample is injected into the vessel, resulting in an increase in exogenous OUR until complete oxidisation of the sludge is reached. The subsequent respirogram is recorded and an alarm is raised if OUR is low (Vanrolleghem et al., 1996).

The STIPTOX incorporates an immobilised turbulent bed of microbes, growing on the inner surface of small hollow cylinders. This is option A for the setup. Option B is a bioreactor containing biomass from the ASP. Sample continuously passes through the culture, and the respiration rate is monitored. The microbes are protected against total inhibition through dilution of the sample ensuring microbes are always available for analysis. The dilution factor along with the reduction in respiration rate is used to return a toxicity value in the unit of percentage inhibition (Envitech, 2005a).

The BOD of wastewater can be measured by the standard ISO 5815-1 method. This standard test is applicable to wastewater with a BOD greater than or equal to 3 $mg.L^{-1}$ with acceptable accuracy up to 6000 $mg.L^{-1}$ (International Standards Organisation, 2003). Whilst the off-line method takes 5 days to complete due to incubation requirements, a commercially available on-line monitor for the estimation of short term BOD (BODst) called Ra-BOD (by AppliTek) takes unfiltered samples through a fast loop system, for direct analysis. The respiration rate is monitored using a single DO probe. Measurement is undertaken at the same temperature and pH as the ASP, allowing close replication of the conditions (AppliTek, 2010). The RODTOX is also capable of estimating BODst, using a similar method to the Ra-BOD (Kelma, 2011).

The ODM-100 oxygen demand monitor (Challenge Technology, USA) is designed for continuous monitoring of the oxygen demand in wastewater. It can be mounted almost anywhere in the WwTW, and provide an early warning of toxicity. It operates by measuring the oxygen supply rate required to maintain a constant oxygen partial pressure in the headspace above the measurement cell. The measurement cell is supplied with a mixture of biomass, and the influent wastewater. Both the influent and biomass are pumped into the cell continuously, or intermittently, allowing either continuous measurement (for toxicity and trend monitoring), or semi-batch measurement (for OUR fingerprint analysis). The OUR is calculated by conducting a mass balance of oxygen input and wastewater flow. The oxygen uptake due to the biodegradation can be calculated by subtracting background endogenous respiration from the total oxygen uptake, giving a measurement of BODst, and a subsequent estimation of the five day BOD (Challenge Technology, 2001; PRWeb, 2011). There are no published application examples of this monitor.

Respirometric devices including the RODTOX, STIPTOX, ODM-100 and Ra-TOX are suited to monitoring acute and chronic toxic events (Table 3.2). If implemented in an EWS, these devices have the potential to reduce the overall risk of an acute toxic event by 12 points and a chronic event by 4 points (Table 3.3).

Table 3.3 Respirometric monitoring devices toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	3
Overall toxic event risk score	**8**	**12**
Reduction of risk score	**12**	**4**

b) Biosensors monitoring metabolic or catabolic inhibition by bioluminescence

Bioluminescence is the emission of light by a living organism, directly proportional to the metabolic activity of the luminescent organism (Lei et al., 2006; Rensing and Maier, 2003). The luminescence of the organism is repressed in the presence of a toxic substance and thus the absence of light can be used as an indicator of toxicity (Ren, 2004). For wastewater toxicity monitoring, the bacterial luminescence lux gene has been widely employed either in an inducible or constitutive manner. The inducible method allows quantitative analysis of the toxic substance concentration. This is achieved by fusing the lux gene to a promoter, regulated by the concentration of the toxic substance (Belkin, 2003; Lei et al., 2006; Rensing and Maier, 2003). Monitoring devices typically involve a culture of bioluminescent bacteria, a reaction cell and a light detector. The idea of utilising a bioluminescence on-line device for wastewater toxicity monitoring was proposed in the early years of the 21st century (Bock Gu and Cheol Gil, 2001; Lajoie et al., 2002; Ren and Frymier, 2003). Whilst more sensitive than a respirometry assay or wastewater flocs, it can be used to screen pollutants or trade effluents. It offers the advantage of being suitable for specific pollutants as well as for a mixture of pollutants that (when combined) can have a toxic effect.

The Microtox® assay has been used for decades as an off-line method for wastewater monitoring, and is the most common strategy in the field of bioluminescence (Love and Bott, 2000). The assay was developed by Azur Environmental (USA) and is based on a naturally occurring luminescent marine bacterial culture known as *Vibrio fischeri*. Recently, an on-line instrument based on the same principle as the off-line test has been

developed, namely the Microtox continuous toxicity monitor (Microtox CTM) (AppliTek, 2010).

Bioluminescence devices including the Microtox CTM are again suited to monitoring acute and chronic toxic events (Table 3.2). If implemented in an EWS, these devices have the potential to reduce the overall risk of an acute toxic event by 12 points and a chronic event by 4 points (Table 3.4).

Table 3.4 Bioluminescence monitors toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	3
Overall toxic event risk score	**8**	**12**
Reduction of risk score	**12**	**4**

c) Biosensors monitoring nitrification inhibition (liquid phase)

These devices are respirometers specifically focussed on analysis of nitrifying bacteria OUR. The devices output an inhibition percentage as a function of OUR reduction, in the same way as traditional respirometers. Like respirometric devices, they typically consist of a measurement cell, a DO probe and a sample feed pump. The OUR of the sample is calculated over a fixed period through the differential in liquid phase DO concentration. An inhibition percentage is subsequently returned.

Nitrifying bacteria are particularly sensitive to variations of influent composition (Jönsson, 2000; Jönsson et al., 2001; Love and Bott, 2000). By their very nature, nitrifiers are slow reproducers and a toxic shock further reduces the replenishment rate (Gerardi, 2002). Hence, monitoring influent toxicity to nitrifiers shows obvious advantages as the basis of an EWS.

The Nitritox Monitor® (LAR process analysers, Germany) uses a 4 litre side-stream fermenter vessel, home to a culture of nitrifying bacteria. The manufacturer recommends using their culture of nitrifying bacteria, along with growth powder but the reactor can also be seeded with AS from the target ASP. Ideal pH (7.6) and temperature (29°C) conditions for nitrifier development are automatically regulated. During sample analysis, a 5 ml portion of nitrifying bacteria is dosed into the measurement cell, leaving

the bulk of the culture un-touched by the sample. It is the respiration rate of these nitrifiers that provides the basis of the monitoring technique.

Liquid phase nitrification monitors such as the Nitritox are suited to monitoring both acute and chronic toxic events (Table 3.2). If implemented in an EWS, this device has the potential to reduce the overall risk of an acute toxic event by 12 points, and a chronic event by 4 points (Table 3.5).

Table 3.5 Liquid phase nitrification monitors (Nitiritox specific) toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	3
Overall toxic event risk score	**8**	**12**
Reduction of risk score	**12**	**4**

3.3.2 Gas monitors

Headspace gas monitors include any monitor that samples non-invasively through the gas phase. Through their normal metabolic activity, microorganisms generate gases that can be emitted to the headspace in the sewer and measured by gas monitors. As such, changes in gas production could be linked to stress responses to toxicity.

It has been shown that N_2O production occurs as a result of nitrification inhibition through denitrification of NO_2- by AOB (Kim et al., 2010). This can occur in the presence of a toxic inhibitor concentration (Butler et al., 2009; Colliver and Stephenson, 2000), making N_2O detection a strategy for toxicity detection at ASPs (Burgess, Colliver, et al., 2002; Burgess, Stuetz, et al., 2002; Butler et al., 2005). Provided nitrifying organisms are present in the sewer biofilm, monitoring N_2O concentration in the sewer headspace gas could potentially be a suitable early warning of nitrification failure at the works (Black et al., 2014).

The N-Tox monitor (Water Innovate, UK) is a commercially available N_2O monitoring device. The gas analysing column employs non-dispersive infrared absorption, relying on the specific absorption spectrum of N_2O. In its current application for toxicity detection at ASPs, the sample is collected directly above the sewage via a floating gas collection hood (Butler et al., 2009). A resolution of 0.1 ppm is available, allowing implementation into environments with low baseline emissions. The detector auto

calibrates the N_2O concentration with respect to temperature, pressure, infrared source ageing and instrumental drift. Owing to a gas filter and semi permeable membrane drier prior to analysis by the gas column, the monitor has a tolerance for 100 % atmospheric humidity (Water Innovate, 2011).

Similarly, it has been shown to be possible to use the off-gas CO_2 mole fraction to monitor nitrification performance in the sewer (Leu et al., 2010). Autotrophic nitrifiers fixate CO_2 (Oguz et al., 2006), and under stress, they would consume less CO_2, resulting in higher concentrations in the headspace. Likewise, methanogens consume CO_2 and generate methane (CH_4) and are also sensitive to toxicity (Capone et al., 1983; Sanchez et al., 1996). This highlights the advantage of employing a mixed culture biofilm as part of an EWS as the response of multiple species to a toxicant could be characterised with the same parameter (i.e. CO_2).

The Click! System (Gas Data, UK) is a modular based biogas analyser. The device can readily monitor CH_4, CO_2, H_2S, and NH_3 gas concentrations through non-dispersive infra-red analysis. The technology is similar to the N-Tox, and it is fitted with gas conditioning equipment allowing high humidity tolerance. As such, it may be possible to modify the gas collection system, for in-sewer placement. The OdaLog® RTx Logger is a commercially available on-line H_2S monitor. Excessive H_2S concentration in the headspace gas within the sewer results in problems with corrosion and odour (Bowker et al., 1991; Jiang et al., 2011). The monitor communicates via a built in GSM modem to a dedicated internet server every 30 minutes. The end user can then access the data collected by their monitor via the internet from any PC with the OdaStat-G software (supplied with the monitor) installed on it. The built in memory can store 42,000 readings with an additional unlimited memory capacity at the central server, allowing data logging to occur every second. An added advantage of the monitor is its battery power source (with a 12-month continuous use supply). This, in conjunction with its compact dimensions (76 mm x 260 mm cylinder) allows utilisation of the monitor anywhere in the sewer network (App-Tek, 2011).

In addition to the gases generated by indigenous biological activity, changes in wastewater composition have been detected with sensor arrays, and they have been shown to offer reliable and reproducible results in the monitoring of wastewater

composition (Dewettinck et al., 2001; Stuetz et al., 1999a, 1999b). Sensor arrays have been shown to be capable of differentiating between different wastewater types and it has been demonstrated that the application of a flow-cell for sensor array analysis of wastewater is practically possible. The analysis of the headspace gas with an electronic nose, can be adequate to determine the quality of wastewater (Bourgeois and Stuetz, 2000). The MS1100 (Multisensor systems Ltd, Cheadle, UK) is a commercially available example, and can detect trace volatile organic carbon (VOC) concentration in wastewater. The system can operate with or without a sampling sidestream tank depending on the application (Multisensor Systems, 2012).

Gas monitors such as the N-Tox, Click!, OdaLog® RTx and MS1100 are suited to monitoring acute toxic events, but it is not likely they will detect chronic events (Table 3.2). If implemented in an EWS, these devices have the potential to reduce the overall risk of an acute toxic event by 12 points (Table 3.6). Due to the nature of monitoring the native biomass response to toxicity, and the wide variability in feed quality, it is likely that sub-lethal toxicant concentration will go undetected within the background variability.

Table 3.6 Gas analysis devices toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✗
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	4
Overall toxic event risk score	**8**	**16**
Reduction of risk score	**12**	**0**

3.3.3 Voltammetry

Accumulation of heavy metal content in wastewater can be toxic to the biological stage of the WwTW. In addition, if heavy metals are still present in the WwTW effluent they can have severe consequences for the accepting surface water environment and public health. Hence, the determination of trace amounts of heavy metals in the influent to the WwTW may be beneficial. This may be possible with an on-line anodic stripping voltammetry (ASV) system.

The electrochemical technique of ASV is for speciation analysis of heavy metals in wastewater, specifically lead, mercury, arsenic and cadmium. The heavy metals in solution are electroplated onto an electrode, concentrating the metal. This is followed by sequential stripping of the heavy metals off the electrode, generating an electrical current (proportional to the amount of a metal stripped off), and allowing quantification of a metal. The metal can also be identified by the characteristic voltage at which it is stripped off the electrode (Kiptoo et al., 2004).

The OVA series (Cogent Environmental, UK) is a 3 electrode device using a glassy carbon mercury thin film electrode. If the device is used to analyse for arsenic, selenium or mercury, a gold electrode can be used (Cogent Environmental, 2011). The voltammetric process analyser (VPA; AppliTek, Nazareth, Belgium) is similar and capable of analysing for up 6 heavy metals at a time, through 6 separate sample streams (AppliTek, 2010). Since voltammetry monitors can detect a broad scale of toxicity upstream of the treatment process, they are suited to monitoring acute and chronic toxic events (Table 3.2). If implemented in an EWS, these devices have the potential to reduce the overall risk of an acute toxic event by 12 points and a chronic event by 4 points (Table 3.7).

Table 3.7 Voltammetry monitors toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	3
Overall toxic event risk score	**8**	**12**
Reduction of risk score	**12**	**4**

3.3.4 Wet chemistry monitors

a) Toxic organic and inorganic compounds wet chemistry monitors

Inorganic compounds are very common in wastewater, mainly from the widespread use of chemical disinfectants and cleaning products. These substances can have undesirable effects at the WwTWs and to the accepting watercourse, including nitrification inhibition (Butler et al., 2009). The EPA (environment process analyser) is a successful commercially available on-line monitor for determination of a very broad range of organic / inorganic compounds in wastewater matrices using proven wet chemistry

methods, offered by AppliTek. The device needs to be configured with the correct standard addition method for the required single parameter to be measured and is capable of analysing three separate samples simultaneously (AppliTek, 2010).

Wet chemistry monitors focussed on organic / inorganic toxicant detection have the potential to reduce the overall risk of a chronic toxic event by 4 points and an acute event by 12 points (Table 3.8). This is owing to a wide toxicant response range and the ability to detect low levels of toxicity.

Table 3.8 Toxic organic / inorganic compound wet chemistry monitors toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	2	3
Overall toxic event risk score	**8**	**12**
Reduction of risk score	**12**	**4**

b) Wastewater sanitary pollutants wet chemistry monitors

If a wet chemistry device monitors wastewater sanitary pollutants it could be employed downstream of the treatment process to give an indication of performance trends.

The chemical oxygen demand (COD) of a wastewater sample can be determined by the standard ISO 6060 method as long as the COD is ≤700 $mg.L^{-1}$ and the chloride content is ≤1000 $mg.L^{-1}$. It is also recommended to dilute wastewater when chloride concentration is >700 $mg.L^{-1}$ (International Standards Organisation, 1989). A commercially available on-line COD monitor is the AppliCOD offered by AppliTek. This monitor operates in compliance with ISO 6060 method, with a built in digestion unit. The response time of this monitor is 20 minutes in comparison to the standard 2-hour digestion method; however, because of the digestion step it is a batch reactor rather than a continuous monitor.

The dissolved and total organic carbon (TOC) concentrations of wastewater can be determined by the standard ISO 8245 test method (International Standards Organisation, 1999). A commercially available TOC on-line monitor is the AppliTOC offered by AppliTek. The device takes filtered batch samples through a self-cleaning setup.

As such, these devices could give an indication of a chronic toxic event, characterised by a downward performance trend. They would potentially reduce the risk of a chronic

event by 4 points (Table 3.9). However, they would not be suited to application upstream of the treatment process and could potentially miss an acute toxic event.

Table 3.9 Sanitary pollutant wet chemistry monitors toxic event risk reduction scorecard.

	Acute toxic events	Chronic toxic events
Toxic event detection	✗	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	5	3
Overall toxic event risk score	**20**	**12**
Reduction of risk score	**0**	**4**

3.3.5 Solid state monitors

a) Solid state dissolved oxygen (DO) and pH monitors

Environmental parameters such as DO and pH are not typically used as stand-alone indicators of toxicity but can be complementary for more complex systems (e.g., respirometers). The determination of the DO concentration of wastewater is possible using an electrochemical cell with the ISO method 5814 describing the standard setup of a DO probe (International Standards Organisation, 2008a). The cell is isolated from the sample by a gas permeable membrane. Another commonly used method employs a luminescent DO probe such as the LDO (Hach Lange, Germany).

For each ammonium ion oxidised in the nitrification process, two protons are released, resulting in a reduction in pH. This can be exploited as a method of nitrification monitoring in poorly buffered systems. In addition, pH can be monitored to analyse the anoxic denitrification process (Gernaey et al., 1997). The pH of wastewater can be determined by the standard ISO 10523 method, in the range of pH 2 to pH 12. The ionic strength of the sample must not exceed 0.3 mol kg^{-1}, with a conductivity of <2000 mS m^{-1} at 25ºC, and the temperature of the sample should be in the range of 0ºC to 50ºC (International Standards Organisation, 2008b). A common method for recording pH on-line is the use of a Hach-Lange pHC probe, accompanied by a Hach-Lange SC1000 data logger.

Monitors of DO and pH are suited to monitoring acute toxic events, but it is not likely they will detect chronic events (Table 3.2). As with headspace gas monitors, they monitor the native biomass response to toxicity. It is not likely they will reduce the risk

score by any more than 4 points (Table 3.10), as a change in DO and pH is not a guaranteed response to toxicity. This would be the case for a nitrification inhibition monitoring application in a mixed culture, where nitrifiers occupy a significantly lower portion of the biomass than heterotrophic bacteria (Tchobanoglous et al., 2014c). In that case, it would be possible that a significant change in DO or pH would not be observed. Also, due to the wide variability in feed quality, it is likely that sub-lethal toxicity will go undetected within the background variability so detection of a chronic event is unlikely.

Table 3.10 DO & pH monitors toxic event risk reduction scorecard.

	Acute toxic events	**Chronic toxic events**
Toxic event detection	✓	✗
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	4	4
Overall toxic event risk score	**16**	**16**
Reduction of risk score	**4**	**0**

b) Solid state sanitary pollutant monitors

Monitoring of nitrogen species allows nitrification performance quantification through a nitrogen balance. The Stipscan system (ISCO-STIP, Germany) can measure NH_3-N as can the Spectron (ISCO-STIP, Germany) a spectrophotometric cabinet analyser (Envitech, 2005b). There are also ion selective process probes available that can monitor NH_4^+-N, NO_3^--N and NO_2^--N such as the AN-ISE-sc (Hach Lange, Germany). Additionally, dissolved N_2O can be monitored online using the N_2O-R electrode (Unisense A/S, Denmark) and has been used to monitor full-scale ASP's (Aboobakar et al., 2013). Since a toxic event could only be detected in the effluent stream of the treatment process, characterised by poor removal performance of sanitary pollutants, these devices are only suited to detecting chronic events (Table 3.2). If implemented in an EWS, they have the potential to reduce the overall risk of a chronic toxic event by 4 points (Table 3.11). Since they monitor the effluent stream, they can only give an indication that a chronic event is occurring, and not provide an early warning. Hence, it is not likely they will reduce the overall risk score by any more than 4 points, as they cannot mitigate the severity of a chronic event to the same extent as a device monitoring the influent. This is the same reason why they cannot detect an acute event, as the point of detection is too late to mitigate the severity.

Table 3.11 Solid state sanitary pollutant monitors toxic event risk reduction scorecard.

	Acute toxic events	Chronic toxic events
Toxic event detection	✗	✓
Likelihood of event (1-5)	4	4
Adjusted severity of event (1-5)	5	3
Overall toxic event risk score	**20**	**12**
Reduction of risk score	**0**	**4**

3.3.6 Emerging technologies

There are a number of monitoring techniques that have not been implemented at full scale, and are hence considered emerging technologies.

Flow injection analysis (FIA), developed in 1979, is a versatile analytical technique based on colorimetric analysis. The method comes with the added benefits of low reagent consumption, good reproducibility, short preparation time and low susceptibility to contaminants (Pashkova et al., 2009). An in-field instrument consisting of a microbial biosensor in a special flow through cell based on FIA for the detection of nitrification inhibitors has been proven at laboratory scale (no commercial development to date). The system can be used for detection of sum parameters (pH, DO and ammonia concentration) and toxicity detection (König et al., 1998).

Titrimetric nitrification monitoring, is based on an acid-base titration and is designed for monitoring ASP's (Hoque et al., 2011). A sample of mixed liquor is taken into the reaction vessel, fed with a known concentration of NH_4^+-N for a set period and maintained at a pH set point through base dosing (e.g. NaOH). The concentration of base added gives an indication of the number of protons released by nitrification and hence the nitrification performance of the sludge (Gernaey et al., 1997).

Calorimetry can be utilised in both aerobic and anaerobic conditions. This is the process of monitoring heat released by microbial communities during conversion of wastewater organic loads. When properly calibrated to BOD data, the calorimetric measurements can give a good indication of BOD concentration on-line (Weppen et al., 1991). This could also be applied to give an indication of microbial activity, i.e. if a sudden change in heat release is detected, the biomass may be under stress.

Flow Cytometry (FCM) is designed to quantify bacterial numbers, and produce multi-parameter data for individual cells in large populations (Amann et al., 1995, 1996;

Forster et al., 2003; Jönsson, 2000). This could be a useful tool for the on-line characterisation of a nitrifying culture, to intricately monitor treatment performance (Bond et al., 1999; Daims et al., 2001; Gieseke et al., 2001; Larsen et al., 2008). It is however limited to laboratory scale at present, but if linked to a sidestream biomass reactor, could give an indication of bacterial numbers and hence the level of inhibition exhibited by a wastewater.

3.4 Future recommendations for wastewater quality monitoring

A Kepner – Tragoe analysis has been conducted, scoring the technologies identified above in Section 3.3 against a set of weighted criteria for acute and chronic toxic event detection. The relative weights of each of the criteria were defined by consulting the key project stakeholders and are summarised in Table 3.12.

Table 3.12 Summary of Kepner Tragoe analysis criteria and weighting.

Criteria	Description/characteristics	Weighting
Toxic event risk reduction	• The most important criteria for an EWS is that it reduces the risk of a toxic event.	50%
Sampling method	• Non-invasive detection is preferable for an in-sewer monitor • Any device requiring sample pre-treatment scored low • A sensor in contact with the liquid phase is permissible at the inlet works	10%
Sensitivity	• Scored high if device can respond to a wide range of toxicant types • Chemical / toxicant specific devices scored low	10%
Response time	• Response time includes the time taken to draw a sample, analyse it and return a response. • If sample pre-treatment is required, devices were scored low.	10%
Maintenance frequency	• The consumable requirements were taken into consideration • Devices were scored low if sample pre-treatment was required • Devices scored low if the bulk test assay comes into contact with the sample, as it will require re-seeding post shock	10%
Housing protection rating	• Important for in-sewer application due to remote sites and security issues • Less important at the inlet works • Monitors were scored according to their most suitable location (i.e. Headspace gas analysis is suitable for in-sewer application due to non-invasive sample technique, liquid phase monitors are suitable for inlet works)	5%
Connectivity	• Scored high if the monitor is compatible with asset standard SCADA systems	2.5%
Power source	• It is important that the device can be readily connected to asset standard power source (110-230 V AC). Devices with this capability scored high. • Additionally, devices with a long life battery also scored high	2.5%

The highest attainable score was 1000. The information used to score the devices was taken from manufacturer data sheets. This score was then compared to the device's cost in 2012 Great British Pounds (£GB) through the following relationship:

$$Points\ per\ £GB = \frac{Score}{Cost} \qquad \textbf{(3.1)}$$

From this, conclusions on the cost effectiveness of a particular technology have been drawn up (Figure 3.3). The N-Tox scored the highest for acute toxicity detection as a result of its non-invasive sampling technique resulting in a lower risk of sensor fouling when compared to liquid phase respiration inhibition, nitrification inhibition, voltammetric, solid state and wet chemistry based monitors. For chronic toxicity detection, the Nitritox monitor scored highest (Figure 3.3), mainly as the bulk culture does not come into contact with the sample and it can respond to a broad range of nitrification inhibitors.

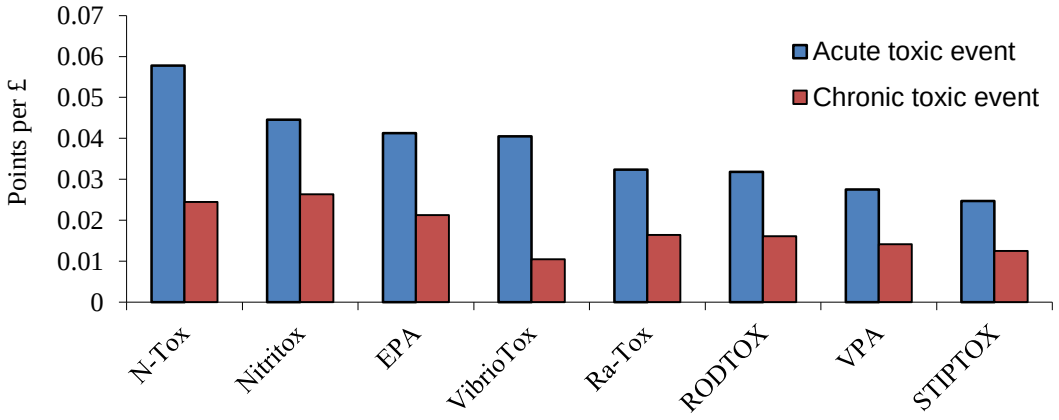


Figure 3.3 Cost effectiveness of commercially available monitoring technologies. Data displayed as cost (£, based on figures from 2012) per point in the Kepner Tragoe analysis.

Since it has been highlighted that DO and pH monitors would potentially be ineffective at reducing the risk of a toxic event (Table 3.2; Table 3.10), they were excluded from the Kepner-Tragoe analysis.

Biosensor devices focussing on respiration and nitrification inhibition are DTA monitors. I.e., rather than focussing on a single parameter or substance type, the toxicity of the wastewater is characterised by a percentage inhibition to the micro-organisms through OUR analysis. Hence, the monitor's response has been compared to standard

inhibition tests. The effective concentration exerting a 50 % inhibition (EC_{50}) on the test culture of the monitoring devices has been collected for a range of known respiration and nitrification inhibitors (Table 3.13). This data is then compared with the ISO 8192 standard respiration inhibition test (International Standards Organisation, 2007) and ISO 9509 standard nitrification inhibition test (International Standards Organisation, 2006). The subsequent correction factor is calculated, in an aim to bring the response of each monitor into the same numerical index by:

$$C_F = \frac{Standard\ EC_{50}}{Monitor\ EC_{50}} \quad \textbf{(3.2)}$$

Table 3.13 EC_{50} Response (R in $mg.L^{-1}$) and Correction factor (CF) in comparison to the relevant ISO standard test (in $mg.L^{-1}$) (Pagga et al., 2006); selected monitors with available data include RODTOX (Kong et al., 1993), Microtox CTM (Modern Water, 2016), Nitritox (LAR Process Analysers, 2016) and AMTOX (Hayes et al., 1998).

Substance	ISO 8192	RODTOX		Microtox CTM		ISO 9509	Nitritox	
		R	CF	R	CF		R	CF
3,5-Dichlorophenol	3.00	11.70	0.26	-	-	2.00	-	-
Allylthiourea	0.70	-	-	-	-	0.50	0.15	3.33
Copper, Cu^{2+}	-	11.4	-	-	-	1.20	-	-
Cyanide	-	0.78	-	>2.50	-	0.34	-	-
Ethanol	>1000	-	-	-	-	>1000	4100	>0.24
Methanol	150	-	-	-	-	>1000	1250	0.24
Nickel, Ni^{2+}	-	-	-	-	-	8.20	-	-
Phenol	0.4	-	-	>20	>0.02	0.80	15	0.05
Zinc, Zn^{2+}	380	-	-	-	-	200	7.5	26.67

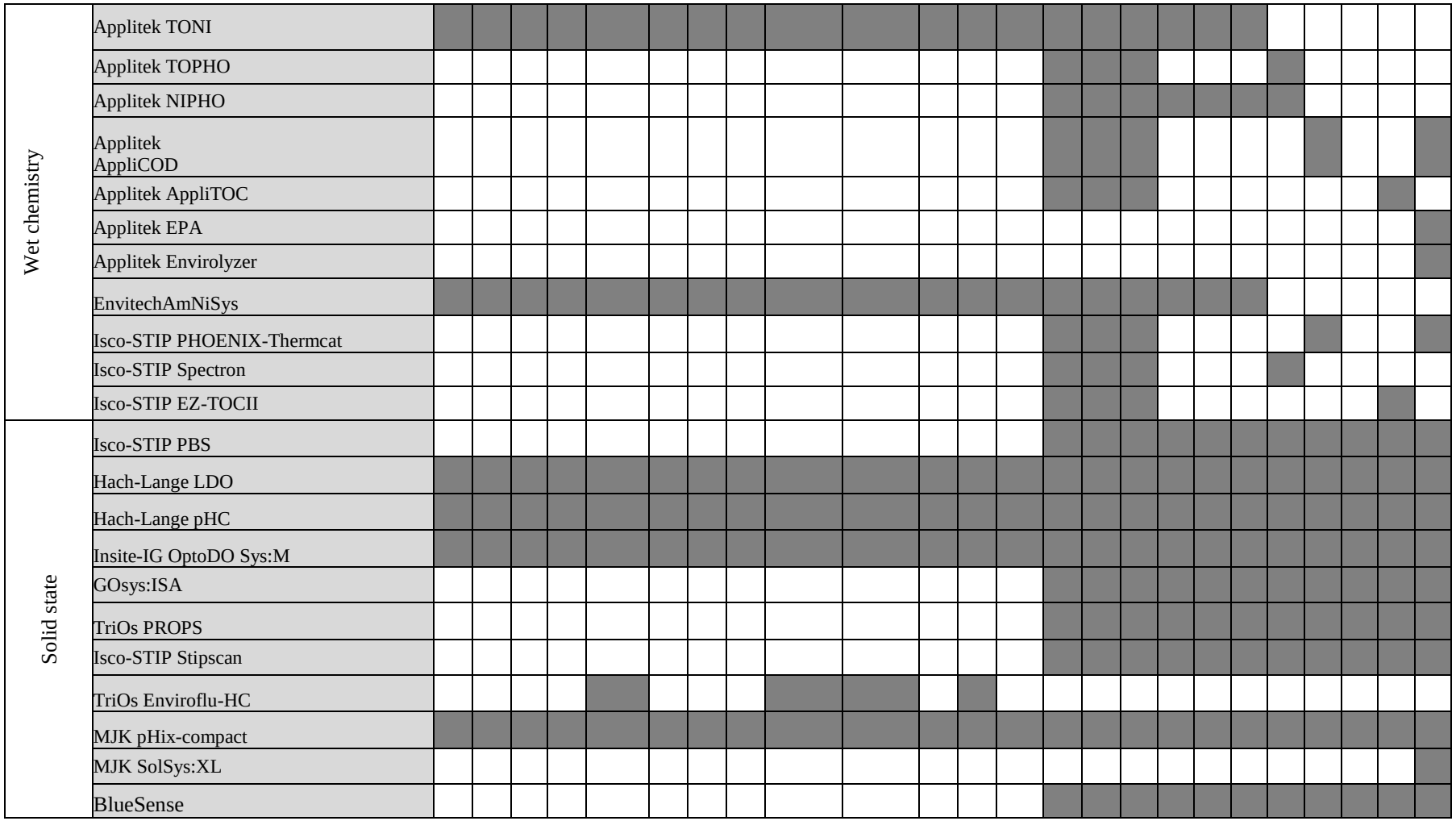
The wide range of inhibitory substances and loading parameters that can influence the response allows application of DTA monitors to complex wastewater compositions (Table 3.14). In comparison to batch chemical analysis methods, the combined effect of all the components present in the sewage on the biological system is taken into consideration, reducing the number of false alarms. For a monitor in this category to be successful, the accuracy of its response is key, in order to characterise the behaviour of the sewer biofilm. The nature of micro-organism cultures employed in the monitoring device is accountable for this response, be it AS, enriched nitrifying culture or *Vibrio fischeri*. All of the DTA monitors performed similarly in terms of correction factors, with the exception of the bioluminescence-based Microtox CTM.

There is some controversy in the use of the V*ibrio fischeri* assay as the basis of wastewater toxicity monitoring (Ricco et al., 2004). It has been reported that *Vibrio*

fischeri are more sensitive to toxic substances than AS. Furthermore, the requirement for salinity and pH adjustment may alter the toxicity of the wastewater sample, thus, a *Vibrio fischeri* culture may not be ideal for monitoring wastewater toxicity in the sewer network (Bock Gu and Cheol Gil, 2001; Gutiérrez et al., 2002; Kong et al., 1993; Lajoie et al., 2002; Love and Bott, 2000; Ren and Frymier, 2003; Salanitro et al., 1988). Academic research incorporating this on-line monitor is non-existent, with the only response testing being conducted by Modern Water (Table 3.13). The correction factor required to bring the EC_{50} response in-line with the ISO 9509 test shows the assay employed cannot be compared to a typical AS culture, possibly leading to false responses in full scale sewer use.

Table 3.14 Monitoring capabilities of devices and factors affecting response highlighted (EWS substance response range) in relation to sewer biofilm response, flow and sediment mobilisation. EC_{50} Values for AS respiration and nitrification inhibition (Pagga et al., 2006), in addtion to typical inhibitory values for loading parameters are displayed.

		Inhibitory substances													**Loading parameters**										
		2,3 Dichlorophenol	**2,4 Dichlorophenol**	**3,5-Dichlorophenol**	**4-Chlorophenol**	**Acetone**	**Allylthiourea**	**Copper (II)**	**Cyanide**	**Ethanol**	**Methanol**	**Nickel (II)**	**Phenol**	**Zinc (II)**	**DO**	**pH**	**Temperature**	**NH_4^+-N**	**NO_3^--N**	**NO_2^--N**	**PO_4^--P**	**COD**	**BOD**	**TOC**	**Suspended solids**
	Activated sludge EC_{50}			3	0.5		0.7			>1000	150		0.4	380	0.1										
	Nitrifier EC_{50}	0.1	0.2	2	0.5	>500	0.5	1.2	0.3	>1000	>1000	8.2	0.8	200		7.6									
Bio-sensors	Challenge ODM-100																								
	Kelma RODTOX																								
	Isco-STIP STIPTOX																								
	Applitek Microtox CTM																								
	LAR Nitritox																								
	Applitek Ra-BOD																								
	Isco-STIP Biox1010																								
Gas monitors	Water innovate N-TOX																								
	Gas Data Click!																								
	App-Tek OdaLog RTx																								
	Multi-sensor MS1100																								
Voltam-metry	Cogent OVA series																								
	Applitek VPA																								


Wet chemistry
Applitek TONI
Applitek TOPHO
Applitek NIPHO
Applitek AppliCOD
Applitek AppliTOC
Applitek EPA
Applitek Envirolyzer
EnvitechAmNiSys
Isco-STIP PHOENIX-Thermcat
Isco-STIP Spectron
Isco-STIP EZ-TOCII
Solid state
Isco-STIP PBS
Hach-Lange LDO
Hach-Lange pHC
Insite-IG OptoDO Sys:M
GOsys:ISA
TriOs PROPS
Isco-STIP Stipscan
TriOs Enviroflu-HC
MJK pHix-compact
MJK SolSys:XL
BlueSense

The low cost effectiveness of DTA monitors (Figure 3.3), and the fact that these systems are prone to sensor fouling, brings the need for a non-invasive technique (Bourgeois et al., 2001; Butler et al., 2009; Love and Bott, 2000; Pedersen and Petersen, 1996). Through monitoring OUR in the gas phase, the ODM-100 may be capable of fulfilling this need, however, the sample system is still very much invasive. Additionally, the device does not hold a supply of a microorganism culture (such as AS) on-board, instead, requiring a constant / intermittent input of fresh AS. This may limit it to a location close to the ASP. For in-sewer application, the monitor would need a pipeline supply of AS from the WwTW. The time taken for the AS to reach the monitor may result in an assay un-characteristic of the actual AS at the works, increasing the likelihood of false positive or negative alarms.

The N-Tox aims to overcome the sensor fouling problem through nitrification monitoring via the gaseous emission of N_2O. This approach is already in existence for monitoring an ASP to give a warning of gradual nitrification failure (Callister et al., 2006). A link has been identified between ammonia concentration in the sludge (as a result of nitrification failure) and the concentration of N_2O in the headspace gas (Burgess, Colliver, et al., 2002; Burgess, Stuetz, et al., 2002). Emissions of N_2O have been recorded in a combined sewer in Germany (Clemens and Haas, 1997), possibly suggesting that the N-Tox can be implemented in the sewer. Likewise, utilising CO_2 as an indicator of nitrification performance (Click! Monitor) is equally cost-efficient as N_2O. These systems do require careful calibration and adequate modelling (with respect to the effect of mass transfer limitations and liquid phase chemical equilibria) (Aulenta et al., 2002). This is due to super-saturation of dissolved CO_2 as a result of a pH alteration. Dissolved CO_2 acts as a pH buffer, shifting the fraction between carbonic acid and bicarbonate, resulting in consumption or release of hydrogen ions (Leu et al., 2010). At standard pH, the concentration of CO_2 in the gas phase is a function of the concentration of dissolved CO_2, alkalinity and pH. With this in mind, this class of monitor is more sophisticated than OUR respirometers. In support of this, the range of inhibitory substances and loading parameters that can influence the response of both the N-Tox and Click! systems match that of the traditional OUR devices (Table 3.14). In keeping with headspace gas analysis, The OdaLog® RTx is a cost effective sewer monitoring technique (Figure 3.3). It is specifically designed for on-line H_2S monitoring

within the sewer (giving an indication of sewer corrosion and odour problems) (Bowker et al., 1991) with a compact size, low power requirements and water proof status (App-Tek, 2011). However, its substance response range as a component of an EWS is very limited (Table 3.14).

The N-Tox, Click! and OdaLog® RTx monitors focus on gas emissions as a response to changing wastewater conditions, without the ability of identifying concentrations of specific compounds in the liquid phase (i.e. they are chemically unspecific). Electronic noses still deliver a non-invasive technique, but have the ability to discreetly monitor concentrations of compounds in the liquid phase. The individual sensor responses (using sensor arrays) have been correlated with the standard diurnal flow to the WwTW, and show repeatability in these responses (Bourgeois and Stuetz, 2002). The addition of a chemical pollutant (e.g. volatile organics such as diesel, toxic to nitrifiers) is translated to a change in response of the sensor array (Bourgeois and Stuetz, 2002). From the sensor profiles, the length of time and the concentration of pollutant in the wastewater can be characterised, meaning that an electronic nose (i.e. the Multisensor MS1100) may prove to be a valuable component of an EWS (Persaud et al., 1996). With correct calibration, it may be possible to predict flow into the works and mobilised pollutant concentration from the sewer sediment. However, the range of inhibitory substances and loading parameters that can influence the response is limited (Table 3.14).

3.4.1 Device selection for acute toxic event detection

Monitoring devices based on headspace gas analysis offer good sensitivity at present, are generally more compact than DTA monitors and have low maintenance intensity (due to lower sensor fouling, and no consumable requirements). With this in mind, and utilising a device selection decision tree (Figure 3.4) devices such as the N-Tox and Click! are well suited for an in-sewer monitoring system, for acute toxicity detection. Similarly, the Nitritox is capable of responding to a broad spectrum of toxicity, and since the bulk culture does not come into contact with the sample, it could be implemented as part of an EWS upstream of the treatment process (for example at the inlet works). It is however not suitable for an in-sewer application, due to the risk of sensor fouling and high maintenance frequency (Figure 3.4).

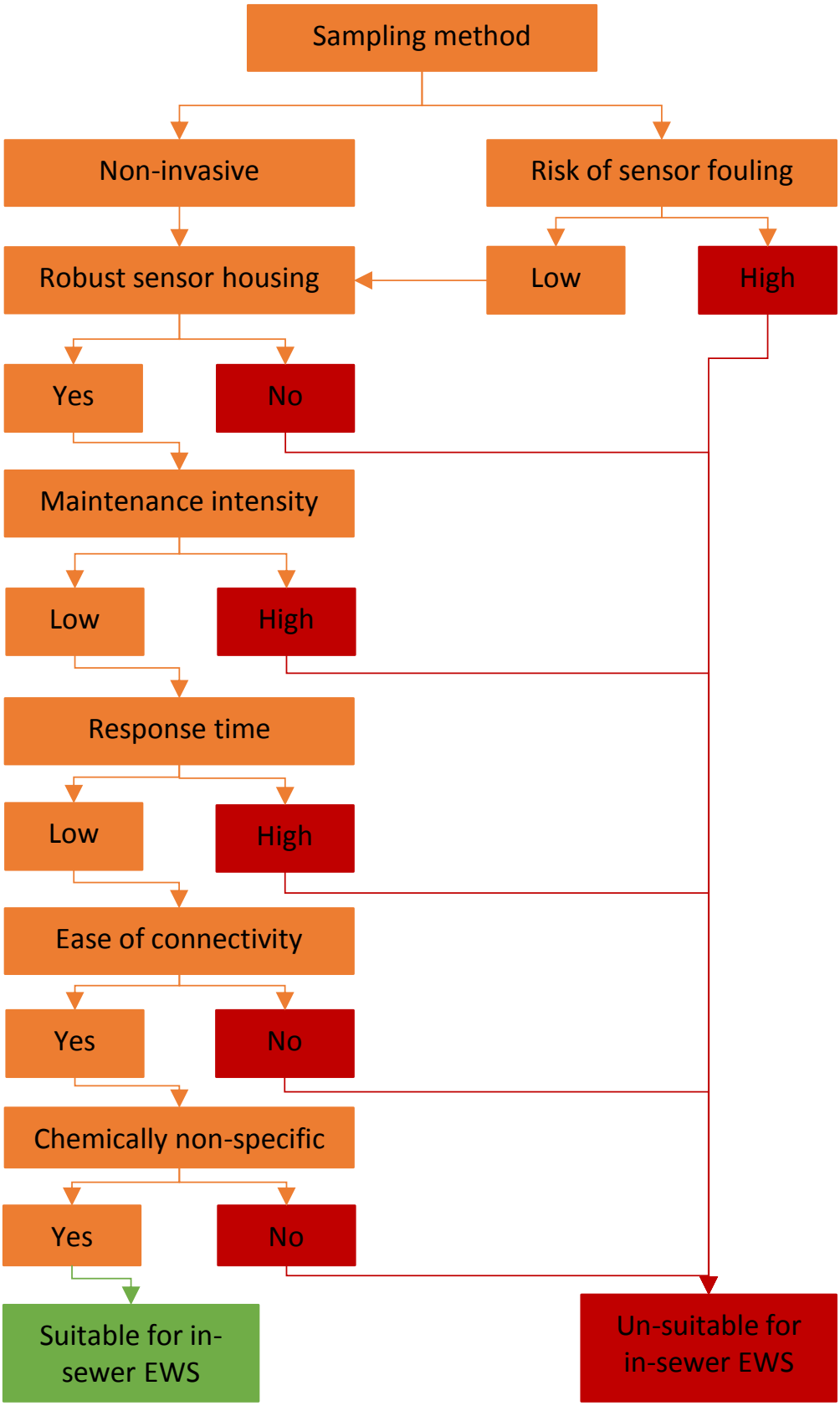


Figure 3.4 Device selection decision tree. Note; decisions on sensor fouling risk, robustness, maintenance intensity, response time and ease of connectivity should be made in line with the criteria descriptions in section 3.2.2.

In-situ monitoring methods such as DO and pH probes are the most cost effective as the range of inhibitory substances and loading parameters that can influence the response matches that of sophisticated DTA monitors and headspace gas nitrification monitors. However, a change in DO and pH is not always a guaranteed response to toxicity, in particular for nitrification inhibition monitoring. As previously mentioned, nitrifying bacteria occupy a significantly lower portion of the sewer biofilm than heterotrophic bacteria (Tchobanoglous et al., 2014c), so it would be possible that a significant change in DO or pH would not be observed.

3.4.2 Device selection for chronic toxic event detection

It is likely that sub-lethal toxicity (leading to a chronic toxic event) would go undetected where devices monitoring responses of the native biomass are employed. This is a result of the wide variability in feed quality, meaning differentiation between the response to low levels of toxicity and the natural background variability would be difficult. Hence, headspace gas monitors should not be employed for chronic event detection.

For an EWS, monitoring toxicity to nitrifying bacteria, a mixed system incorporating N-Tox devices in the sewer and a Nitritox at the inlet works would offer a robust EWS solution, capable of detecting acute and chronic toxicity. Again, as the bulk culture is not in contact with the sample, and its response range is broad, the Nitritox is well suited to chronic toxic event detection. The N-Tox devices would provide an early warning of acute toxicity and the Nitritox at the inlet works would rationalise the response, and verify if the wastewater is toxic on an acute or chronic scale.

Thus, application of the N-Tox as an in-sewer EWS device and Nitritox as an inlet works monitor have been explored in a detailed study (CHAPTER 4). The response of the sewer biofilm to toxicity has been profiled through toxic shock testing, and modifications have been applied to adapt the N-Tox for in-sewer use.

CHAPTER 4. DEVELOPMENT OF AN IN-SEWER EARLY WARNING SYSTEM

4.1 Phase I: Identifying a suitable monitoring device

Based on the literature review (CHAPTER 3), two nitrification inhibition monitors were evaluated in a laboratory environment namely, the Nitritox (LAR) and the N-Tox (Water Innovate). The response of the monitors was tested against ATU, a known nitrification inhibitor.

4.1.1 Nitritox

A Nitritox monitor and autotrophic nitrifying culture was obtained from LAR Process Analysers AG, Berlin, Germany. The operational process of the Nitritox is described in section 3.3.1c. Settled sewage samples, obtained from Severn Trent Water's Finham WwTWs in Coventry UK, were spiked with ATU concentrations of 0.5 $mg.L^{-1}$, 1.0 $mg.L^{-1}$, 1.5 $mg.L^{-1}$, 2.0 $mg.L^{-1}$, 2.5 $mg.L^{-1}$, 3.0 $mg.L^{-1}$, 3.5 $mg.L^{-1}$ and 4.0 $mg.L^{-1}$. The measurement cell was flushed with 100 ml clean tap water before each test. A 10 ml portion of continuously stirred sample was then drawn into the measurement cell using the built in peristaltic pump followed by a 10 ml portion of oxygen saturated clean tap water. The OUR of the continuously stirred mixture was then measured over a 2 minute period, and taken to be the baseline OUR. Next, an electronic actuated valve allowed a 5 ml portion of the nitrifying culture into the measurement cell, driven by the static head in the 5 litre fermentation vessel. The OUR of the continuously stirred mixture was measured over a 2 minute period.

The OUR of the 5 ml portion of nitrifying culture was calculated as:

$$\mathbf{OUR_2 - OUR_1 = OUR_3} \qquad \mathbf{(4.1)}$$

Where; OUR_1 = OUR of sample and clean water

OUR_2 = OUR of sample, water and nitrifying portion

OUR_3 = OUR of nitrifying culture

The device periodically measured the OUR of the bulk nitrifying culture and compared this to the OUR measured for the sample culture (OUR_3). From this, the percentage inhibition during the sample run was calculated as:

$$\textbf{percentage inhibition} = \frac{\mathbf{OUR_3}}{\mathbf{OUR_0}} \times \mathbf{100} \qquad \textbf{(4.2)}$$

Where; OUR_0 = OUR bulk nitrifying culture

Each sample test was repeated twice, to test the reliability of the monitor's response. Following analysis, the measurement cell was automatically flushed with a 5 %w/v detergent / water solution.

To validate the monitor's response, the specific nitrification rate of the Nitritox culture was measured off-line. A 100 ml sample of the culture was placed in a 200 ml conical flask. To this, 50 ml of settled sewage was added and NH_4^+-N determined at time t = 0 and t = 60 minutes using standard methods (see section 4.5). The volatile and fixed suspended solids concentrations (VSS and FSS) were determined using Standard Methods (APHA, 2005).

4.1.2 N-Tox

An N-Tox monitor was obtained from Water Innovate, Cranfield, UK. The operational process of the N-Tox is described in section 3.3.2. For the N-Tox analysis, a pilot scale sewer test rig was designed and built as described in section 4.3.1. Biofilm was grown for 13 days in a sewer biofilm pipe under the flow and level conditions as described in section 4.3.1. A 10 litre batch quantity of settled sewage from Severn Trent Water's Minworth WwTW (replenished daily) was circulated through the system. On day 14 the batch was spiked with 0.5 $mg.L^{-1}$ and 2.0 $mg.L^{-1}$ of ATU (prepared as described in section 4.4.1), which correspond to literature values for EC_{50} and EC_{75} concentrations of ATU (Pagga et al., 2006), and circulated for 90 minutes. Gaseous N_2O emissions were monitored for 24 hours, after which the batch was replenished with fresh settled sewage, circulated and monitored for a further 24 hours.

4.2 Phase II: Establishing the minimum requirements for sewer deployment of an EWS device

To establish the minimum requirements for sewer deployment and gain understanding of the conditions an EWS would need to be resistant to, the N-Tox was deployed to a full-scale sewage pumping station. The sewer system chosen as a case study was the Strongford network (Severn Trent Water, Stoke-on-Trent, UK), which conveys domestic, commercial and industrial wastewater to a 342,000 PE WwTW in Stoke-on-Trent, UK. The Chemical Lane sewage pumping station in Stoke-on-Trent was chosen as the analysis site due to ease of access, security, standard electricity supply and its location on an industrial wastewater route. Baseline N_2O emissions in the headspace above the wastewater level within the wet well were analysed for a period of 27 days in August 2011 (Figure 4.1). In addition, DO, pH and temperature data was collected using LDO and pH-D process control probes (Hach-Lange, Salford, UK) and logged using an SC100 controller (Hach-Lange, Salford, UK).

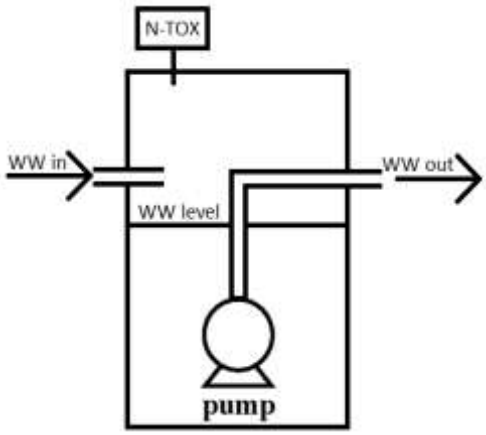


Figure 4.1 Sewage pumping station wet well setup.

4.3 Phase III: Biofilm development in the pilot scale sewer and CFBBR systems

4.3.1 Pilot scale sewer design and operational set-up

A pilot scale rig (Figure 4.2) was designed / constructed and consisted of six pipes (5 test pipes and 1 standby pipe) for development of a sewer biofilm over 13 days (Black et al., 2014). The pipes were fed continuously with fresh settled sewage transferred to the test rig by pump from the ASP distribution chamber at Severn Trent Water's 2.2 million PE Minworth WwTW in Birmingham, UK. The conditions in all pipes were

controlled with uniform flow rate, level and feed sewage composition. During the biofilm development period the baseline un-inhibited conditions were monitored in each pipe, and compared against each other. Conditions continued to be monitored during toxic shock tests and compared across all pipes, where the rig allowed 3 conditions to be tested as follows:

1. The biofilm in pipes 1 & 2 was developed for EC_{50} toxicity response tests;
2. The biofilm in pipes 3 & 4 was developed for EC_{75} toxicity response tests;
3. The biofilm in pipe 5 was developed as a control that was not exposed to toxicity during toxic shock tests.

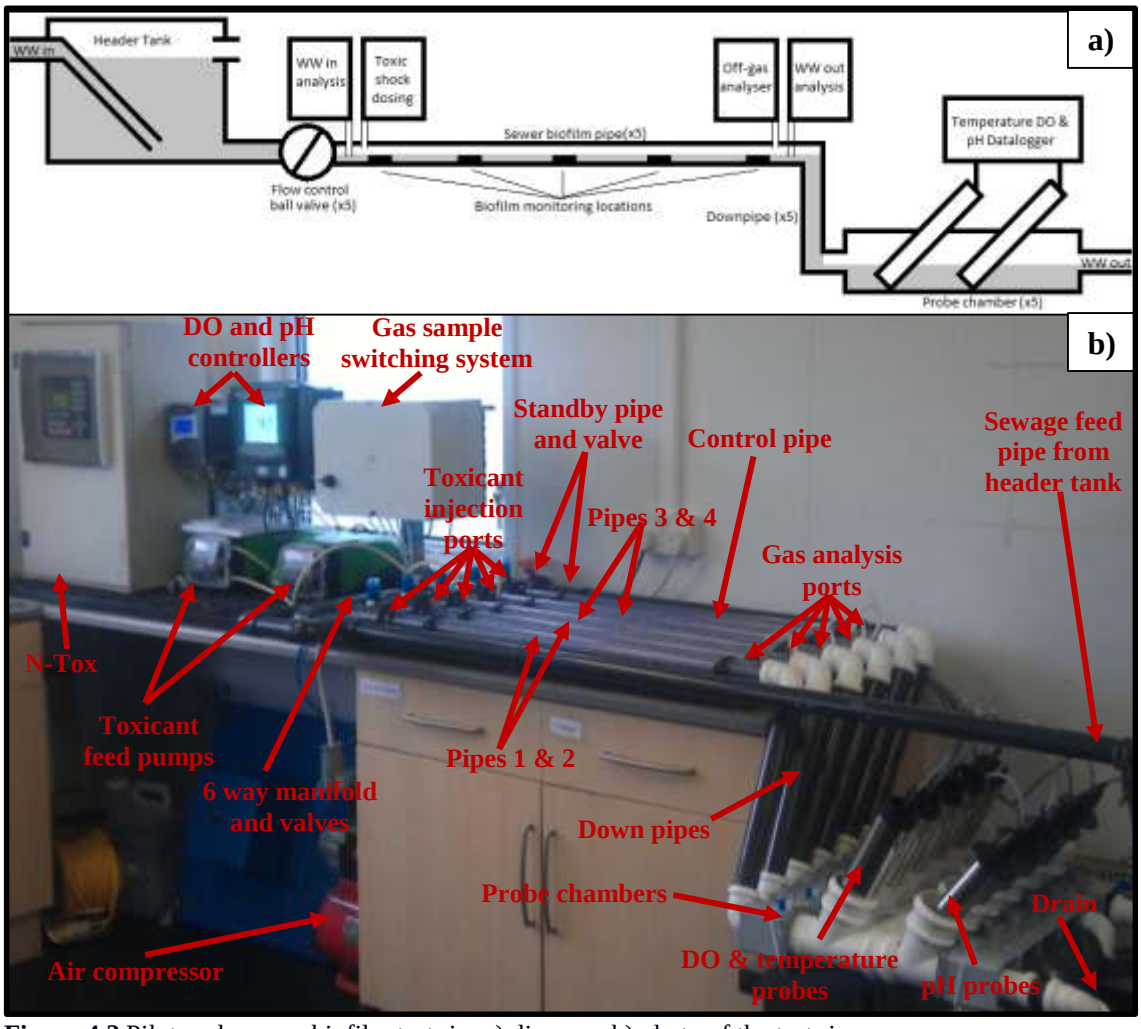


Figure 4.2 Pilot scale sewer biofilm test rig, a) diagram, b) photo of the test rig.

As the valves were solvent welded to the manifold and therefore not removable, pipe 6 was provided as the standby in-case of valve failure on the 5 test pipes.

The biofilm test pipes were constructed using pipes, fittings, valves and solvent cement from EPCO Ltd., Leeds, UK. All joints between pipe sections and fittings were solvent welded.

A six-way manifold was constructed from 40 mm diameter opaque PVC pipe, 4 tee sections and a 90º bend joined sequentially along the straight length. To the open orifices of the 90º bend and the 4 tee sections, ball valves were fitted. The valves were graduated to provide precise control of flow to the biofilm test pipes. The remaining open orifice of the manifold was connected to a 60 litre header tank (Figure 4.2a).

The test pipes were formed from 40 mm diameter (36 mm inner diameter) clear PVC pipes cut into 1000 mm lengths. Six of these in total were made and were joined to the 5 ball valves. A 6 mm hole was drilled in the top of the test pipes at the start to form the toxic shock port. At the end of the test pipes, a 6mm hole was drilled and fitted with a 6 mm push fitting for connection to the N-Tox (Figure 4.2b).

A 90º bend was joined to the opposite end of each biofilm pipe. To these bends, 300 mm long down pipes (formed from 40 mm diameter opaque PVC pipe) were fitted. The bottom of the down pipes was fitted with 90º bends and connected to 75 mm to 40 mm reducer fittings for connection to the probe chambers (Figure 4.2a).

The probe chambers were formed of two 75 mm diameter 45° tee sections joined sequentially on the straight lengths. The 45° angled orifices of the probe sections were faced upwards allowing a chamber for the DO and pH probes to fit into. Two 6 mm holes were drilled in the top of the probe chambers, directly above the sensing end of the probes. Flexible 6 mm diameter tubing was passed through these holes and connected to the compressed air system. A pneumatic timer switch (RS Components, Stockport, UK) was fitted to the compressed air supply to the probe chambers, providing air scour of the probes every 10 minutes (Figure 4.2b).

A 75 mm to 40 mm reducer fitting was fitted to the end of the probe chambers to effectively form a liquid retention depth of 18 mm. This ensured the probes were continually wet. To the end reducers of the probe chambers, 90º bends with tails were fitted and flexible 40 mm diameter hosing joined to the tails. This formed the effluent route to the drain (Figure 4.2b).

There were five evenly spaced locations down the length of the pipes, each with four coupons (one for each characterisation day) on both sides of the pipes (a total of eight at each location and 40 in each pipe) to allow for replication (Figure 4.2a). The coupons fitted snugly into bespoke 8 mm holes spaced at 12 mm centre to centre and matched the pipe wall thickness and curvature. This ensured a smooth join and minimal disruption to the wastewater flow. The centre of each hole was 8 mm high, in respect to the inner base of the pipes. A 15 mm × 11 mm × 2 mm (height × width × thickness) plastic support plate with identical curvature to the outer pipe wall was solvent welded to each circular coupon. The outer perimeter of the coupons and the inner surface of the plates were coated in polytetrafluoroethylene (PTFE) gel (Screwfix Direct Ltd., Somerset, UK) and a tight strap held the coupons in place, forming a water tight bond.

Settled sewage was continuously fed to the header tank by a Flygt Ready 4 submersible pump (Xylem Water Solutions, Nottingham, UK) located in the ASP distribution chamber. Settled sewage then gravitated from the header tank to the biofilm pipe manifold and into each of the biofilm pipes. Each biofilm pipe acted as a gravity fed sewer line, conditioning a natural biofilm on the wall of the pipes. Biofilm nitrification activity was monitored at the 5 monitoring locations and an initial steady state in activity was observed after 13 days. As mentioned in section 3.2, the wide range of solids sizes and time varying flows in a real sewer disturbs the biofilm at the water sediment interface. As such, the sewer biofilm is not expected to reach full maturity, and to simulate this, toxicity was monitored on very young biofilms.

A constant 25 % cross-sectional area capacity in each pipe was maintained with a flow and level of 1.0 $L.min^{-1}$ and 8.5 mm respectively, allowing a biofilm growth area along the 1 metre pipe length of 0.028 m^2. Sewage velocity was 0.07 $m.s^{-1}$ resulting in an HRT of 14 seconds.

Gaseous N_2O emissions were monitored on-line using an N-Tox monitor. A compressed air actuated timer switching system (Air Engineering Controls Ltd., East Sussex, UK) automatically switched the sample gas stream to the N-Tox monitor between each biofilm pipe every 2 minutes. The 2 minute gas monitoring period on each biofilm pipe consisted of a 1 minute purge stage and 1 minute of emissions logging giving a resolution of 10 minutes on each biofilm pipe. Temperature, DO and pH were

monitored on-line in each pipe using LDO and pH-D process control probes (Hach-Lange, Salford, UK) and logged using SC100 and SC1000 controllers (Hach-Lange, Salford, UK). The biofilm was characterised on days 4, 7, 11 and 13 of each conditioning period from the 5 monitoring points as described in Section 4.5 (Figure 4.2a). The volatile and total solids (VS and TS) content of the detached biofilm was measured following Standard Methods (APHA, 2005), and the coupons were returned to the pipes after rinsing with clean tap water post analysis. In addition, on day 13 the specific nitrification rate of the biofilm on eight coupons per pipe was assessed off-line as described in Section 4.5.

4.3.2 CFBBR system

Following the sewer biofilm testing stage described in section 4.4.1, wide variation between the baseline emissions of each pipe was observed. Comparison to the control pipe proved difficult despite identical conditions and was attributed to variability in the biofilm community. Hence, it was deemed appropriate to take pre-shock data for a given pipe as the control for that pipe (i.e. each pipe acted as its own control). The same methodology was applied to the CFBBR biofilm development study.

In the sewer biofilm pipes, it was necessary to conduct biofilm nitrification assays and toxic shock tests in replicate at the "global" pipe scale (i.e. 2 pipes for each condition tested) due to low control of biofilm thickness. The same was not true for the CFBBR systems, where each system consists of 200 biofilm carrier elements.

Polyethylene Kaldnes K3 media (Veolia Water Technologies, West Midlands, UK) was employed as biofilm carrier elements with a diameter of 25 mm, a specific gravity of 0.96, a specific biofilm growth area of 500 $m^2.m^{-3}$ in bulk (Rusten et al., 2000) and a volume of 5 ml per carrier. The design is such that biofilm thickness is controlled by uniform growth surface area on each carrier, and collisions between the carriers. Replication was thus on the carrier element scale and not the "global" reactor scale.

The biofilm development study was conducted in three stages as detailed below:

- **Stage I - Bacterial seeding of the reactor:**
  Reactor R_0 (50 litre cylindrical tank) was filled to 70 %v/v (Table 4.1) with media and fed for 14 days with a continuous fresh feed of settled sewage

transferred to the reactor by pump from the ASP distribution chamber at Severn Trent Water's 2.2 million PE Minworth WwTW in Birmingham, UK.

- **Stage II - Biofilm pre-growth**:

  400 carriers were taken from reactor R_0 and split equally between two CFBBR systems R_1 and R_2 giving a fill fraction of 14 %v/v (Table 4.1). This quantity was determined to be the maximum possible (data not shown) before interruption to flow and blockage of the reactor. For another 78 days, R_1 and R_2 were fed with a nutrient solution designed to enrich the biofilm for nitrifying bacteria (recipe defined in section 4.7.2). To minimise the volume of nutrient solution required, HRT was set at 6 hours. Additionally, reactor R_0 continued to be fed for the same 78 day time period with settled sewage transferred to the reactor by pump from the ASP distribution chamber to sustain an unenriched biofilm.

- **Stage III: HRT testing**:

  The biofilm carriers enriched for nitrifiers in R_1 and R_2 were kept in place for this stage. A further 600 carriers were taken from R_0 and split equally between another three CFBBR's (Reactors R_3, R_4 and R_5). All five CFBBR's were then fed with settled sewage at differing flow rates for 62 days (Table 4.1). Additionally, reactor R_0 continued to be fed for the same period with settled sewage to sustain contingency biofilm.

Following the 3 biofilm development stages described above, reactor R_0 continued to be fed fresh sewage to sustain the culture for the toxicity response testing phase (described in section 4.4.2) at a carrier fill fraction of 60 %v/v. A steady state nitrification rate was reached 180 days after the start of stage I, and all toxicity testing was undertaken after this point.

Table 4.1 CFBBR operating conditions.

Reactor	HRT (min)	Reactor Volume (L)	Fill fraction (%)	Influent concentrations ($mg.L^{-1}$)		NH_4^+-N loading rate ($g.m^{-2}.d^{-1}$)	COD loading rate ($g.m^{-2}.d^{-1}$)
				NH_4^+-N	COD		
Stage I, bacterial seed							
R_0	20	50	70	25 – 41	140 – 290	5 – 8.4	28.8 – 59.7
Stage II, biofilm pre-growth							
R_0	20	50	60	25 – 36	148 – 278	6 – 9.8	33.6 – 69.6
R_1	360	7	14	382*	-	3.8	-
R_2	360	7	14	382*	-	3.8	-
Stage III, flow rate / HRT testing							
R_1	5	7	14	27 – 38	150 – 262	203 – 392	984 – 3306
R_2	10	7	14	27 – 38	150 – 262	101 – 196	492 – 1653
R_3	5	7	14	27 – 38	150 – 262	203 – 392	984 – 3306
R_4	10	7	14	27 – 38	150 – 262	101 – 196	492 – 1653
R_5	50	7	14	27 – 38	150 – 262	21 – 39	99 – 331

*Synthetic nutrient solution with consistent NH_4^+-N content

The CFBBR design was based on previous published studies (Lazarova and Manem, 1996; Lazarova et al., 1997). Five 7 litre CFBBR's were designed and built for this study (Figure 4.3). The reactors were constructed using pipes, fittings, valves and solvent cement from Pipestock Ltd., Romsey, UK. All joints between pipe sections and fittings were solvent welded.

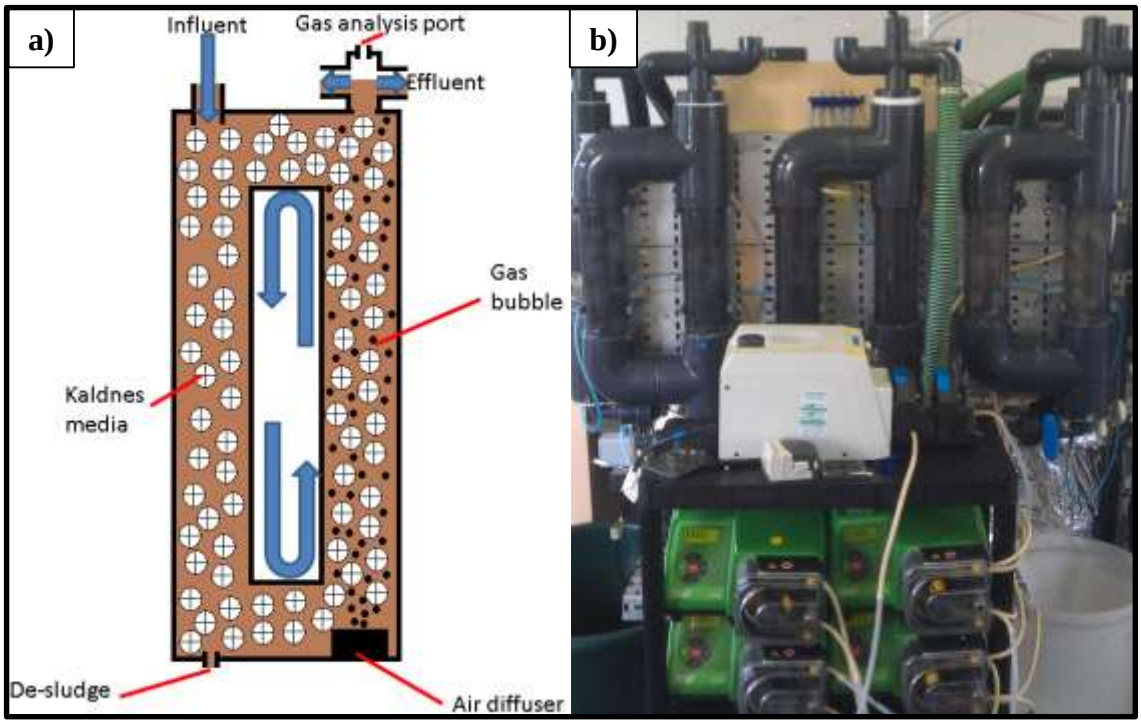


Figure 4.3 Circulating floating bed biofilm reactor; a) diagram of one reactor, b) photo of the test rig – Reactor R_1 is on the rear left side (out of shot) of the rig, R_2, R_3 and R_4 are along the front (left to right) and R_5 is on the rear right side (out of shot).

The reactor riser and down-comer shafts were constructed from 100 mm outer diameter clear PVC pipes. All transparent reactors were covered in aluminium foil, to prevent algal growth. The top and bottom of the down-comer were formed by smooth 90° bend fittings. The top and bottom of the riser shaft was formed from 100 mm diameter tee sections which were then subsequently joined to the 90° bends of the down-comer shaft. This completed the reactor vessel.

The base of the riser shaft was formed by a 100 mm to 25 mm reducer fitting. A ball valve was fitted into the outer face of the reducer fitting for reactor draining and de-sludge. A flexible ethylene propylene diene monomer (EDPM) micropore 60 cm long tube diffuser (Interpet, Surrey, UK) was coiled and fitted to the inner face of the base section, leaving a space in the middle to allow free passage of liquid during draining. A flexible 6 mm diameter tube was passed through a 6 mm diameter hole in the base section and connected to the diffuser. Once completed, the base section was coated with PTFE gel around the joint surface, slotted into the base of the riser bottom t-section and secured with 6 hex screws around the joint. The latter allowed easy removal for servicing and cleaning.

The top of the riser shaft was formed by a 100 mm to 40 mm reducer fitting. A 40 mm cross (4 way) fitting was joined to the reducer fitting. To the left and right orifices of the cross fitting, 90° bends with tails were joined and flexible 40 mm diameter hosing fitted to the tails. This formed the reactor effluent route to the drain. A cap was fitted to the top orifice of the cross fitting to form the reactor headspace. A 6 mm hole was drilled in the middle of the cap and a 6 mm push fitting was attached. This allowed connection of the gas analysis equipment and completed the reactor construction.

Aeration in the riser shaft was provided with a Clarke Wiz Mini air compressor (Clarke International, Essex, UK). This was connected to the flexible tube fitted to the diffuser. An airflow meter and pinch valve was fitted between the compressor and diffuser to control airflow to the reactor.

An 8 mm hole was drilled in the top of the down-comer shaft and a flexible 8 mm Tygon peristaltic tube passed through the hole. The reactors were fed with wastewater through this tube using a Watson Marlow 520 S peristaltic pump (Watson Marlow Ltd, Cornwall, UK) set to 0.7 $L.min^{-1}$.

4.3.3 CFBBR biofilm growth, hydrodynamics and mass transfer

The rate of biomass production was minimised to limit over growth of new bacteria such that the concentration of limiting substrate in the influent was roughly equal to concentration in the reactor / effluent (i.e., $s \approx s_i$).

$$\mathbf{m = YQ(s_i - s)} \qquad \mathbf{(4.3)}$$

where; m = biomass production rate, $kg.hr^{-1}$

s_i = influent concentration of limiting substrate, $kg.m^{-3}$

s = effluent concentration of limiting substrate, $kg.m^{-3}$

Y = yield coefficient, $kg_{biomass.}\ kg_{substrate}^{-1}$

Q = Flowrate $m^3.hr^{-1}$

The overall relationship between gas hold-up, apparent solids hold-up and superficial gas velocity was described as reported in the literature (Lazarova and Manem, 1996; Lazarova et al., 1997):

$$\boldsymbol{\varepsilon_g = (10 + 5.54 C_s) U_g^{1.44}} \quad \textbf{(4.4)}$$

where; ε_g = overall gas hold-up, dimensionless

C_s = apparent solids hold-up, %v/v

U_g = superficial gas velocity, $m.s^{-1}$

The superficial gas velocity was set at 0.02 $m.s^{-1}$ and overall solids hold-up was 14.3 %. From this, the volumetric oxygen mass transfer coefficient was calculated as reported in the literature (Lazarova and Manem, 1996; Lazarova et al., 1997):

$$\boldsymbol{k_L a = (1.61 \times 10^3) \frac{\varepsilon_g}{1-\varepsilon_g}} \quad \textbf{(4.5)}$$

where; $k_L a$ = volumetric oxygen mass transfer coefficient, hr^{-1}

ε_g = overall gas hold-up, dimensionless

The saturated DO concentration, taken to be the concentration in equilibrium with atmospheric concentration, was calculated as follows using Henry's law:

$$\boldsymbol{C_{DO}^* = \frac{p}{H}} \quad \textbf{(4.6)}$$

where; C_{DO}^* = saturated DO concentration, $mg.L^{-1}$

p = atmospheric partial pressure of oxygen, atm

H = Henry's constant for oxygen, $atm.L.mg^{-1}$

The partial pressure of oxygen under ambient conditions was taken to be 0.21 atm. Henry's constant for oxygen under ambient conditions was taken as 0.024 $atm.L.mg^{-1}$.

From this, the oxygen mass transfer rate in the reactor was calculated as:

$$\boldsymbol{n = k_L a\,(C_{DO}^* - C_{DO})} \quad \textbf{(4.7)}$$

where; n = oxygen mass transfer rate, $kg.m^{-3}.hr^{-1}$

$k_L a$ = oxygen transfer coefficient, hr^{-1}

C_{DO} = DO concentration in effluent, $kg.m^{-3}$

C^*_{DO} = saturated DO concentration, kg.m^{-3}

4.4 Phase IV: Characterising the biofilms' response to toxic shock event

4.4.1 Response of the sewer biofilm to a toxic event

The development period of the sewer biofilm and its ability to support nitrifiers was characterised using specific nitrification rate assays described in section 4.5.2 on days 4, 7, 11 and 13 of the conditioning period (Table 4.2). The biofilm responses in terms of N_2O emissions and nitrification rates were monitored to test the hypothesis that nitrifiers response to a toxic shock event results in increased N_2O production by the biofilm system.

Table 4.2 Timeline of sewer biofilm development phase, characterisation with nitrification assays and toxic shock testing.

Period	Day														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Biofilm conditioning period	•	•	•	•	•	•	•	•	•	•	•	•	•		
Biofilm characterisation with nitrification assays				•			•				•		•		
90 minute EC_{50} Toxic shock to pipes 1&2														•	
90 minute EC_{75} toxic shock to pipes 3&4														•	
N_2O emissions monitoring in all pipes (including control)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Biofilm N_2O, CO_2, DO an pH toxicity response														•	
Post shock N_2O, CO_2, DO an pH monitoring															•

On day 14 of the conditioning period (Table 4.2), a toxic shock test was conducted with a single toxicant at both an EC_{50} (pipes 1 & 2) and EC_{75} (pipes 3 & 4) based on values reported in the literature (Table 4.3). Whilst EC_{50} values were reported for all toxicants, EC_{75} concentrations were calculated based on the ratio of EC_{50}:EC_{75} reported for ATU toxicity to AS (Pagga et al., 2006).

Each shock test took 15 days to complete, to allow for the biofilm conditioning period and testing of two target concentrations in duplicate. The batch toxicant solutions were prepared with wastewater taken from the header tank and ATU powder (Fisher

Scientific, Loughborough, UK). The shock solution was drip fed at a flow rate of 0.05 $L.min^{-1}$ into the wastewater flow of 1.0 $L.min^{-1}$ for 90 minutes using a Watson Marlow Sci-Q multichannel cartridge pump (Watson Marlow Ltd, Cornwall, UK). Toxicant solution drip feeding was done via the toxic shock ports at the start of the biofilm pipes. The required concentration and volume of the bulk toxicant solution was calculated according to equations (4.8 and 4.9). Post toxic shock, the gaseous N_2O emissions, DO, temperature and pH were monitored for a further 24 hours.

$$\mathbf{V_T = t \times F_T} \quad \textbf{(4.8)}$$

$$\mathbf{C_T = \frac{EC_T \times (F_{WW} + F_T)}{F_T}} \quad \textbf{(4.9)}$$

where; C_T = Toxicant bulk concentration, $mg.L^{-1}$

EC_T = Toxicant effective concentration, $L.min^{-1}$

F_T = Toxicant dose flow rate, $L.min^{-1}$

F_{WW} = Wastewater flow rate. $L.min^{-1}$

t = shock duration, minutes

V_T = Toxicant volume, L

Table 4.3 Tested nitrification inhibitors and their approximate effective 50 % and 75 % concentrations. **Calculated based on ratio of ATU EC_{50} to EC_{75}.*

Compound	Active Toxicant	EC_{50} ($mg.L^{-1}$) (Pagga et al., 2006)	EC_{75} ($mg.L^{-1}$) (Pagga et al., 2006)
Allylthiourea	ATU	0.5	2
Potassium dichromate	Chromium (Cr^{6+})	16	64*
Cupric Sulphate	Copper (Cu^{2+})	1.5	6.0
Nickel Sulphate	Nickel (Ni^{2+})	8.2	33*

4.4.2 Response of the CFBBR biofilm to a toxic event

Reactors R_1, R_2, R_3, R_4 and R_5 were setup to test the toxicity responses of the same substances as in the sewer system. However, based on learning from the sewer biofilm pipe tests, the CFBBR reactors were tested with concentrations determined by a dose response analysis and not reported EC_{50} and EC_{75} literature values (see section 4.5.3). Dose response curves were produced (see section 4.5.3) and the corresponding EC_{50}

concentration for the biofilm was determined for ATU, chromium (VI), Copper (II) and Nickel (II). These concentrations are reported in section 4.6.4a.

The toxicity response testing phase took place after the 3 biofilm development stages (described in section 4.3.2), at least 200 days after the start of the bacterial seed stage (stage I) as nitrification performance was in steady state. For each toxicity test, 200 biofilm carriers with pre-grown biofilm were taken from reactor R_0 (Table 4.4) using a net and placed into one of the CFBBR systems (reactors R_1, R_2, R_3, R_4 and R_5). The biofilm was allowed to acclimatise to the conditions in the CFBBR for 13 days, before being shocked with a toxicant on day 14 with a range of concentrations (Table 4.4), including those determined to totally inhibit nitrification in dose response tests (section 4.5.3). In addition to gaseous N_2O, DO and pH measurements in the effluent, gaseous CO_2 emissions were also monitored for the full test period (Table 4.4) using a GMD20 CO_2 transmitter (Vaisala Ltd, Birmingham, UK). Data was transmitted via 0 – 10 V outputs to a Picolog 1216 voltage logger (Pico Technology, St Neots, UK) coupled with a data logging windows 7 based laptop.

Table 4.4 Timeline of CFBBR biofilm toxicity response testing cycle.

Period	Day														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
200 carriers with pre-grown biofilm taken from R_0 and placed into CFBBR	•														
200 virgin carriers to replace removed carriers	•														
Biofilm aclimitisation period in CFBBR	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Biofilm transfer period in reactor R_0	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
120 minute toxic shock to test														•	
Baseline N_2O, CO_2, DO an pH monitoring in test CFBBR	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Biofilm N_2O, CO_2, DO an pH toxicity response														•	
Post shock N_2O, CO_2, DO an pH monitoring															•

Through trial and error, it was determined that only one CFBBR should be monitored at any one time due to the risk of missing the start of the toxicity response emissions peak. Hence, the pneumatically actuated gas sample switching system was not employed in the CFBBR biofilm toxicity response testing phase and as such, simultaneous shock testing was not undertaken.

Additionally, it was not possible to conduct back to back or even simultaneous shock testing as the 200 carriers removed from reactor R_0 had to be replaced with virgin carrier elements to maintain the 60 %v/v fill fraction. Following this replacement, reactor R_0 was left un-touched for at least 14 days to allow biofilm to transfer to the new carriers (Table 4.4). To ensure this process did not have a significant effect on the performance of the bulk culture, no more than 5% (equating to 307 so the 200 carriers is within the 5% limit) of the biofilm carriers in reactor R_0 were removed and replaced with virgin carriers at any one time. Hence, the 14 day biofilm transfer period in reactor R_0 and the 13 day acclimatisation period in the test CFBBR limited the frequency of toxic shock testing to one every 27 days.

4.4.3 Toxicity response of sewer and CFBBR biofilms sustained with synthetic wastewater

A control toxicity test on a biofilm conditioned with synthetic wastewater was conducted to produce an N_2O emissions baseline under controlled conditions for the sewer and CFBBR biofilms. The synthetic wastewater consisted of 375 $mg.L^{-1}$ glucose, 76.4 $mg.L^{-1}$ NH_4Cl, 22 $mg.L^{-1}$ KH_2PO_4, 300 $mg.L^{-1}$ $NaHCO_3$, 10 $mg.L^{-1}$ $CaCl_2$, 0.823 $mg.L^{-1}$ $FeSO_4.7H_2O$, 0.058 $mg.L^{-1}$ $MnSO_4.4H_2O$, 0.062 $mg.L^{-1}$ $ZnSO_4.7H_2O$, 10 $mg.L^{-1}$ $MgSO_4$ and 50 $mg.L^{-1}$ yeast extract (Fisher Scientific, Loughborough, UK) based on the published recipe by Choung and Kim, 2000. A 3 day supply of synthetic wastewater was maintained, to ensure the biofilm could be fed over the longest un-attended time period (i.e. the weekend).

For the sewer test pipes, the biofilm was conditioned with real wastewater (as described in section 4.3.1) during the initial week to seed the reactors, and fed with a synthetic wastewater solution thereafter. A 90 minute shock test was conducted on day 14 and post shock conditions were monitored for 24 hours after the shock (Table 4.5).

Table 4.5 Timeline of sewer biofilm development phase with synthetic wastewater, characterisation with nitrification assays and toxic shock testing.

Period	Day														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Biofilm conditioning period with continuous fresh real wastewater feed	•	•	•	•	•	•	•								
Biofilm conditioning period with synthetic wastewater								•	•	•	•	•	•	•	•
Biofilm characterisation with nitrification assays				•			•				•		•		
90 minute EC_{50} Toxic shock to pipes 1&2														•	
90 minute EC_{75} toxic shock to pipes 3&4														•	
N_2O emissions monitoring in all pipes (including control)	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Biofilm N_2O, CO_2, DO an pH toxicity response														•	
Post shock N_2O, CO_2, DO an pH monitoring															•

For the CFBBR system, 200 carriers were removed from reactor R_0 and placed in a CFBBR (as described in section 4.4.2). The biofilm was fed with synthetic wastewater throughout the test period (Table 4.6).

Table 4.6 Timeline of CFBBR biofilm (conditioned with synthetic wastewater) toxicity response testing cycle.

Period	Day														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
200 carriers with pre-grown biofilm taken from R_0 and placed into CFBBR	•														
200 virgin carriers to replace removed carriers	•														
Biofilm aclimitisation period in CFBBR fred with synthetic wastewater	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
Biofilm transfer period in reactor R_0	•	•	•	•	•	•	•	•	•	•	•	•	•	•	
120 minute toxic shock to test														•	
Baseline N_2O, CO_2, DO an pH monitoring in test CFBBR	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Biofilm N_2O, CO_2, DO an pH toxicity response														•	
Post shock N_2O, CO_2, DO an pH monitoring															•

4.5 Analytical methods

4.5.1 Water quality analysis for the sewer and CFBBR

Water quality parameters were determined from filtered samples using cuvette test kits and a DR3800 spectrophotometer (Hach Lange, Salford, UK) as described in APHA, 2005. Dissolved COD was determined using LCI400 cuvette tests. The NH_4^+-N concentration was determined using Hach-Lange LCK303 and LCK304 cuvette tests with measuring range of 2 $mg.L^{-1}$ to 47 $mg.L^{-1}$ NH_4^+-N (for the Nitritox influent and biofilm reactor samples) and 0.015 $mg.L^{-1}$ to 2 $mg.L^{-1}$ NH_4^+-N (for Nitritox measurement cell effluent samples). The NO_3^--N concentration was determined using LCK339 and LCK340 cuvette tests with measuring range of 0.23 $mg.L^{-1}$ to 13.5 $mg.L^{-1}$ NO_3^--N (for the Nitritox influent and biofilm reactor samples) and 5 $mg.L^{-1}$ to 35 $mg.L^{-1}$ NO_3^--N (for Nitritox effluent samples). The NO_2^--N concentration was determined using LCK341 cuvette tests with measuring range of 0.015 to 0.6 $mg.L^{-1}$ NO_2^--N.

4.5.2 Specific nitrification rate

A 1 L nitrification assay solution was prepared using the synthetic wastewater recipe (section 4.4.3), and split into 200 ml beakers (one for each pipe or reactor). In the case of the sewer biofilm pipes, 8 coupons per pipe were rinsed with 60 ml of deionised water to remove the biofilm and the mixture was added to the test assay. In the case of the CFBBRs, 8 floating carrier elements were placed directly into the assay. The NH_4^+-N concentration was measured at time t = 0 using NH_4^+-N cuvette test kits (Hach-Lange, Manchester, UK), after which the beakers were aerated for 60 and 180 minutes for pipes and reactor biofilm respectively, maintaining the DO at ~10 $mg.L^{-1}$. At time t = 180 minutes, the aeration was stopped, followed by quantification of the NH_4^+-N concentration (as above). From this, the nitrification rate (NR) was calculated as:

$$\mathbf{NR} = \frac{C_{t=0} - C_{t=60}}{VS.\ t} \qquad \textbf{(4.10)}$$

Where; NR = Nitrification rate, mg-NH_4^+-N.m^{-2}.hr^{-1}

$C_{N(t=0)}$ = Initial ammonium concentration, mg-NH_4^+-N.L^{-1}

$C_{N(t=60\ or\ 180)}$ = Ammonium concentration after 1 hr aeration, mg-NH_4^+-N.L^{-1}

VS = volatile solids concentration on coupons, mg-VS

t = 1 hour

For the biofilm pipes, toxicity was monitored by measuring the inhibited nitrification rate of the biofilm. After measuring the baseline (un-inhibited) nitrification rate of the detached biofilm from each pipe in the 200 ml beakers, the corresponding inhibiting substances were added in concentrations commensurate to those used in the on-line toxic shock tests (Table 4.3). Aeration was resumed for a further 60 minutes. At time t = 60 minutes, the aeration was stopped and NH_4^+-N concentration determined as previously described. From this, the inhibited NR (NR_I) was calculated as per equation 4.9. The percentage inhibition of nitrification was then calculated as:

$$\% \textbf{ inhibition} = \textbf{100} - (\frac{\textbf{100}}{\textbf{NR}} \times \textbf{NR}_\textbf{I}) \qquad \textbf{(4.11)}$$

For the CFBBR reactor biofilm, an un-inhibited control was run simultaneously to the test assays. Nitrification inhibition was calculated in the same way as above, with the nitrification rate of the control being NR.

The above method was also used to construct a dose response curve for the CFBBR biofilm for ATU, copper (II), chromium (VI) and nickel (II).

4.5.3 Dose response curves

To establish the inhibitory effect of known toxicants, dose response tests were conducted for the CFBBR biofilm and sewer biofilm as well as suspended cultures at 10.5 mg-MLSS.L^{-1} (representative of the same solids mass as 8 CFBBR carriers) and 2850 mg-MLSS.L^{-1} (representative of a full scale ASP). Specific nitrification inhibition tests were conducted as described in section 4.5.2, and concentrations of toxicants were added in four-fold increments until a 100 % inhibition was reached. Nitrification inhibition percentage was then plotted against toxicant concentration (log scale) and a dose response curve with non-linear regression was fitted using Prism 6.05 for Windows (GraphPad Software, La Jolla, CA, USA) to determine the EC_{50} concentration. The dose response curves produced for the 3 systems in each condition were then compared against each other using hill slope factors and an F-test. The hill slope factor quantifies the steepness of the dose response curve against the standard curve, i.e. a standard dose response curve has a hill slope of 1, a steeper curve has a hill

slope factor > 1 and a shallower curve has a lower hill slope factor < 1. The dose response curves were also compared with an F-Test, and the resulting p values in the 0.05 significance scale and r^2 values were used to assess the difference in response to toxicity between the 3 systems (i.e. CFBBR biofilm, 10.5 mg-MLSS.L^{-1} and 2850 mg-MLSS.L^{-1}.

4.5.4 Statistical analysis

To validate the monitoring approach, a two-way analysis of variance (ANOVA) followed by a Dunnett's multiple comparison test was conducted at the 0.05 level of significance, comparing the test pipe emissions with the control pipe emissions 180 minutes immediately pre-shock (termed un-inhibited) and 180 minutes immediately post-shock (termed inhibited). A low p value indicated rejection of the null hypothesis, and that emissions were significantly different pre and post shock. The time window was chosen to allow any change post the 90-minute shock to be picked up (i.e. double the shock length) and to discount the high variability in emissions that would have an effect on the mean values over 24 hours, owing to varying influent composition. In addition, to verify that the spike in emissions resulted from the toxic shock, the difference between inhibited and un-inhibited tests was determined for each pipe separately using a two tailed paired t-test. All data were checked for normality prior to analysis and the distribution of residuals checked after the test. All statistical analysis was conducted with Prism 6.05 for Windows. Additionally, a 90-point moving average (reflecting the toxic shock duration) was applied to the emissions data to enhance the time series definition.

To aid change point detection and simplify comparison between tests, cumulative sum (CUSUM) control charts were constructed for the CFBBR reactor biofilm toxic shock events. The method used is reported elsewhere (Hinkley, 1971; Pettitt, 1980; Taylor, 2012) and is intended to be complementary to the emissions time series. The CUSUM charts are designed to confirm significant changes have occurred, rather than pinpoint the exact change point location in time. A negative CUSUM trend indicated data was below the overall average, positive trend indicated data was above the average and a flat line indicated data was around the overall average (Hinkley, 1971; Pettitt, 1980; Taylor,

2012). The sharpness of the CUSUM change point was direct proportional to the statistical significance of the change in raw data.

To quantitatively analyse the CUSUM change point, the slope change was calculated about each CUSUM data point. To do this, the slope for the 10 minutes (one HRT) prior to the data point was calculated as dy/dx, where dy was the change in CUSUM data, and dx was the change in time (10 minutes). The slope change over the 10 minutes after the data point was then calculated. A large positive slope change value indicated a steep CUSUM incline and a large negative slope change value indicated a steep CUSUM decline.

4.6 Results

4.6.1 Phase I: Identifying a suitable monitor

The critical literature review (CHAPTER 3) identified the N-Tox and Nitritox monitors as the two most suitable devices to provide an early warning of nitrification inhibition at the WwTW. The suitability of each monitor was then tested in a pilot sewer lab study. The devices are based on two different detection techniques allowing the sensitivity of detection of a liquid phase monitor (Nitritox) to be compared against a headspace gas phase monitor (N-Tox).

a) Nitritox monitor

Baseline nitrification inhibition detected by the Nitritox sampling ATU-free settled sewage was 14 % and 25 % for replicates 1 and 2 respectively, increasing to 90 % and 92 % respectively, for settled sewage spiked with 4 $mg.L^{-1}$ of ATU (Figure 4.4). Reproducibility in the monitor's response was evident at all concentrations tested (Figure 4.4). The limit of detection of the monitor was determined as 24 %. This implies any programmed toxicity alarm should be set above this value to minimise false positives.

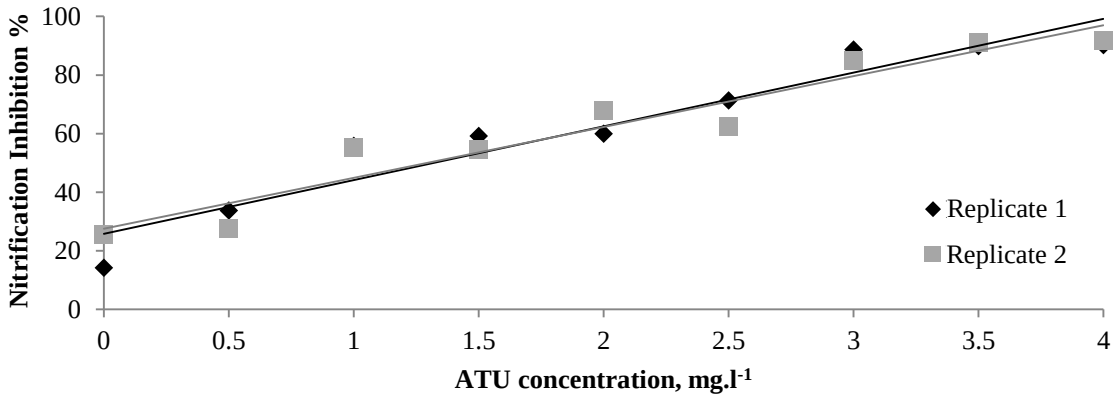


Figure 4.4 Toxicity response of Nitritox culture as a function of ATU concentration.

To validate the Nitritox culture's response to toxicity a comparison was made with reported literature values for full scale suspended growth culutres in ASPs. The Nitritox detected a 34 % and 28 % nitrification inhibition for a 0.5 mg.L^{-1} ATU shock (Figure 4.4) in comparison to a 50% nitrification inhibition reported in the literature for full scale suspended growth cultures (Hayes et al., 1998; Pagga et al., 2006). The device also detected a 60 % and 68 % nitrification inhibition for a 2.0 mg.L^{-1} ATU shock (Figure 4.4) in comparison to 75 % nitrification inhibition reported in the literature for full scale suspended growth cultures (Hayes et al., 1998; Pagga et al., 2006). This highlights a lower sensitivity of the Nitritox culture to nitrification inhibitors in comparison to full scale suspended growth systems.

When examining the wastewater quality before and after passing through the monitor, a significant increase in dissolved COD was observed, from an initial settled sewage concentration of 55 mg.L^{-1} to a final concentration of 315 – 395 mg.L^{-1} (i.e., post nitrification inhibition measurement) for the 2.5 mg.L^{-1} ATU shock test. This suggests the seed from the nitrification culture was COD-rich. Furthermore, the higher inhibition percentage resulted in lower effluent COD values, contrary to what would be expected based on oxygen uptake rates. A potential explanation for this is an inconsistent biomass injection volume between the different tests. This may be attributed to a non-uniform floc size in the reactor vessel, with evidence of biomass clumping and subsequent non-homogenous mixing (Figure 4.5) as a result of routine issues with the nutrient solution and growth powder dosing systems. This would suggest that the monitor's response is dependent on the concentration of biomass present in the

measurement cell, which would need to be considered when implementing the monitor in the field.

Figure 4.5 Biomass clumping (highlighted by red dashed circle) in the Nitritox reactor.

Other issues were identified in addition to biomass clumping. Firstly, evidence of filamentous growth was evident through bulking and foaming in the reactor. Secondly, the measurement cell would often block up (due to its small form factor) and the sample was not always fully flushed from the cell.

From a practical deployment standpoint, the monitor required daily attention to ensure it was working as designed. A revised maintenance schedule was developed which was more onerous than the manufacturer's original recommendations (Appendix 1). Owing to these issues, the Nitritox would be considerably high maintenance if placed at the inlet of a WwTW.

b) N-Tox

The performance of the N-Tox was tested with an 90-minute batch shock test. Emissions of N_2O were found to be relatively stable prior to a shock, with emissions from all pipes following comparable baselines (Figure 4.6). After spiking with 0.5 $mg.L^{-1}$ ATU (EC_{50} concentration) and 2.0 $mg.L^{-1}$ ATU (EC_{75} concentration), N_2O emission from the test pipes increased with increasing ATU concentration, with measurable spikes (with a 6 hour duration) above the control pipe baseline (Figure 4.6).

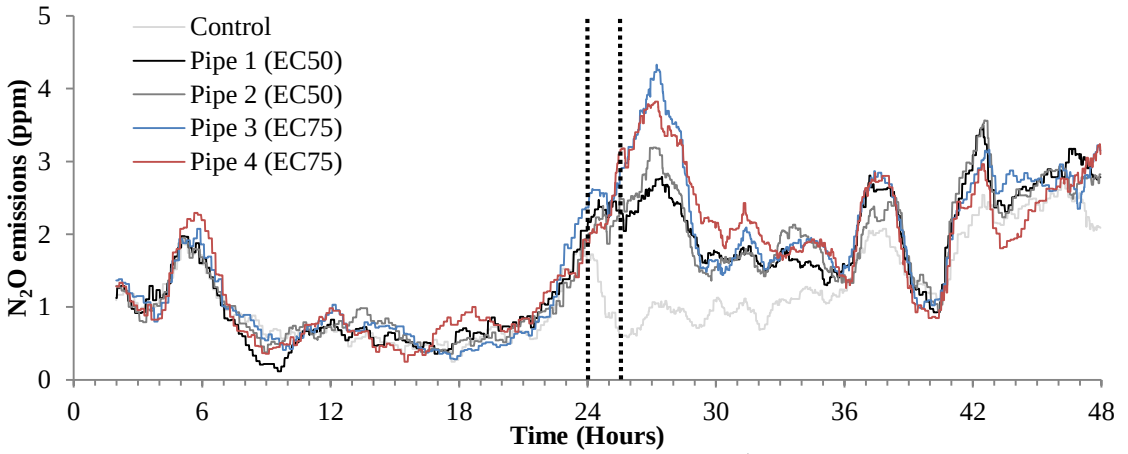


Figure 4.6 Initial response of pipe biofilm to a 90 minute 2 $mg.L^{-1}$ ATU toxic shock at minute 1440 (within black dotted lines).

In terms of practical use of the N-Tox there were some requirements that resulted in monitoring downtime. Firstly, it required calibrating with an N_2O gas standard once every 6 months. Secondly, the diaphragm in the gas sample pump will fail roughly twice per year, due to continual operation. Thirdly, the gas membrane filter needed to be inspected regularily, as depending on how harsh or polluted the gas was with solids, the membrane will foul. Left un-resolved, a fouled gas membrane filter will result in excessive strain on the sample pump, and can lead to premature failure. Finally, the monitor required an annual service, which due to the specialist equipment necessary to carry out the service, had to be done at the manufacturers facility.

This initial off-line study suggested the N-Tox monitor could be used to detect toxicity based on gas analysis in the sewer, expanding from its current application on ASPs.

4.6.2 Phase II: Establishing the minimum requirements for sewer deployment of an EWS device

Theoretically, gravity sewers carrying crude sewage with concentrations of NH_4^+-N around 30 $mg.L^{-1}$ could sustain a nitrifying biofilm (Nielsen et al., 1992; Short et al., 2014). Indeed, averages of 27 $mg.L^{-1}$ NH_4^+-N were monitored in the tested sewage pumping station, along with a favourable pH range of 6.7 to 7.7. However, the measured DO concentrations of 0.6 to 1.0 $mg.L^{-1}$ could limit oxygen flux into the biofilm which can be detrimental to both nitrifying performance (Tchobanoglous et al., 2014a) and the response mechanisms to nitrification inhibitors. Furthermore, the N_2O

emissions baseline from a full-scale sewage pumping station was on average below the limit of detection of 0.5 ppm (Figure 4.7).

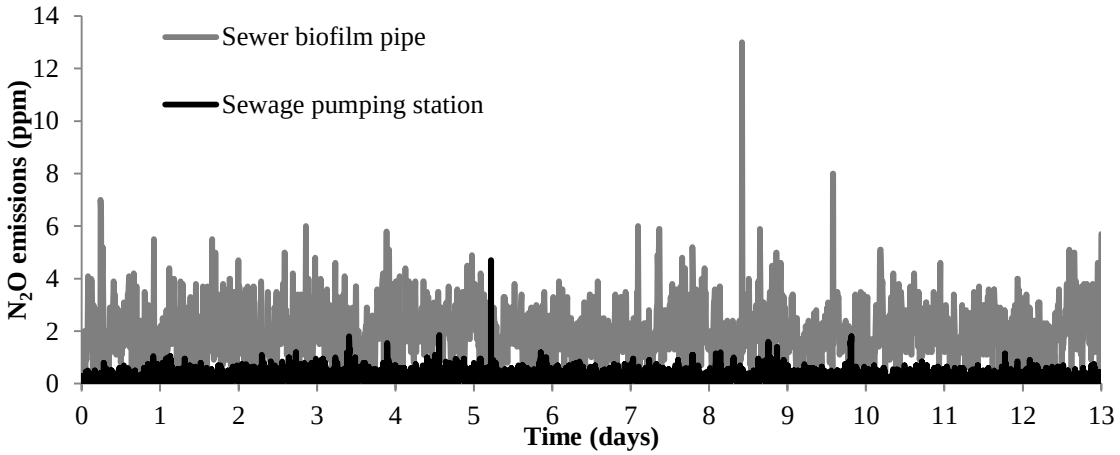


Figure 4.7 Biofilm 13 day N_2O emissions baseline for sewer biofilm tests pipes and the full scale chemical lane sewage pumping station in Stoke-on-Trent, UK.

The harsh sewer environment was evident through the wide range of organic and inorganic suspended solid sizes and configurations including plastics, wood and ragging (Appendix 2), which resulted in probe fouling and pump failures. This, along with time varying flows disturbing the water-sediment boundary, can result in sloughing (Banasiak et al., 2005). An in-depth pilot scale study was conducted, to explore the potential of employing an N-Tox in the sewer.

4.6.3 Phase III: Biofilm development in the pilot scale sewer and CFBBR systems

a) Pilot scale sewer

Over the 13 day conditioning period, VS content in the sewer biofilm pipe system increased to a plateau of approximately 25 g-VS.m^{-2} in all 5 pipes (Figure 4.8). Between day 4 and 7, the VS content increased by 47 %; the plateau was reached by day 7. The biofilm on this day was considered to have reached an initial steady state as the increase in VS over the remaining time accounted for only 6 %. Further confirmation for a steady state at day 7 was obtained from the relative proportion of VS:TS, which barely changed after that day. There was no significant statistical difference in VS average development in all cases (Figure 4.8).

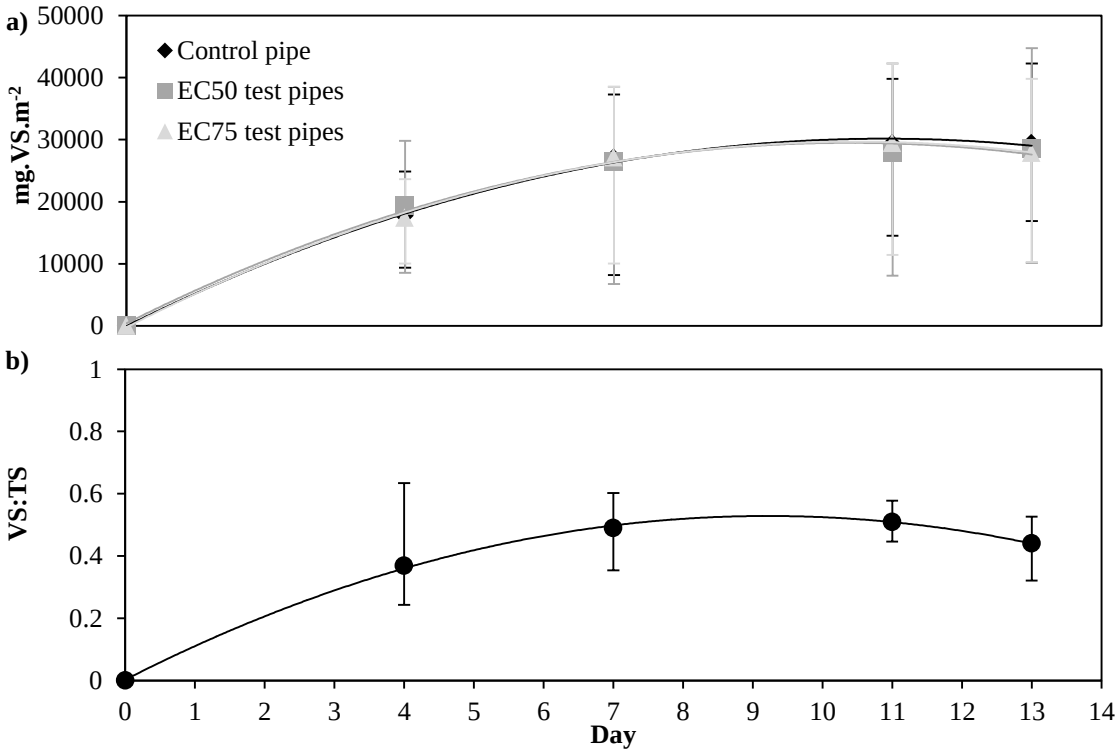


Figure 4.8 Biofilm development during the study; a) Volatile solids development (per m^2). The average of all five conditioning periods is denoted by the trend lines, with the minimum and maximum displayed with range bars; b) VS:TS average ratio for all tests conducted.

The specific nitrification rate of the sewer biofilms throughout the study ranged between 0.38 and 1.34 mg-NH_4^+-N.mg-VS^{-1}.d^{-1}, with an average of 0.78 mg-NH_4^+-N.mg-VS^{-1}.d^{-1} (or 19.5 g-NH_4^+-N.m^{-2}.d^{-1}). This is 0.66 NH_4^+-N.mg-VS^{-1}.d^{-1} higher than the specific nitrification rates of 0.12 mg-NH_4^+-N.mg-VSS^{-1}.d^{-1} (at DO ≈ 5.4 mg.L^{-1}) found in AS systems (Dotro et al., 2011) and higher than specific nitrification rates found in other biofilm systems such as moving bed biofilm reactors (Dulkadiroglu et al., 2005). The specific nitrification rate measurement confirms the presence of a nitrifying population within the pilot-scale sewer biofilm in spite of the short HRT and relatively short growth times when compared against conventional nitrifying systems (Tchobanoglous et al., 2014c).

Measurable N_2O emissions were observed over the 13 day conditioning period, averaging 2.1 ppm (Figure 4.7). This contrasted with reported emissions from nitrifying systems, which are in the range of 16.5 – 186.3 ppm for ASPs (Aboobakar et al., 2013; Butler et al., 2009; Lo et al., 2010), 25 ppm for sequencing batch reactors (Kampschreur, Tan, et al., 2008), and 135 ppm in nitritation reactors (Kampschreur, van der Star, et al., 2008). The low baseline emissions were unexpected, as the sewer

system could nitrify, the influent NH_4^+-N was between 23 - 48 mg.L^{-1}, and the DO was ranging between 1 – 2 mg.L^{-1}.

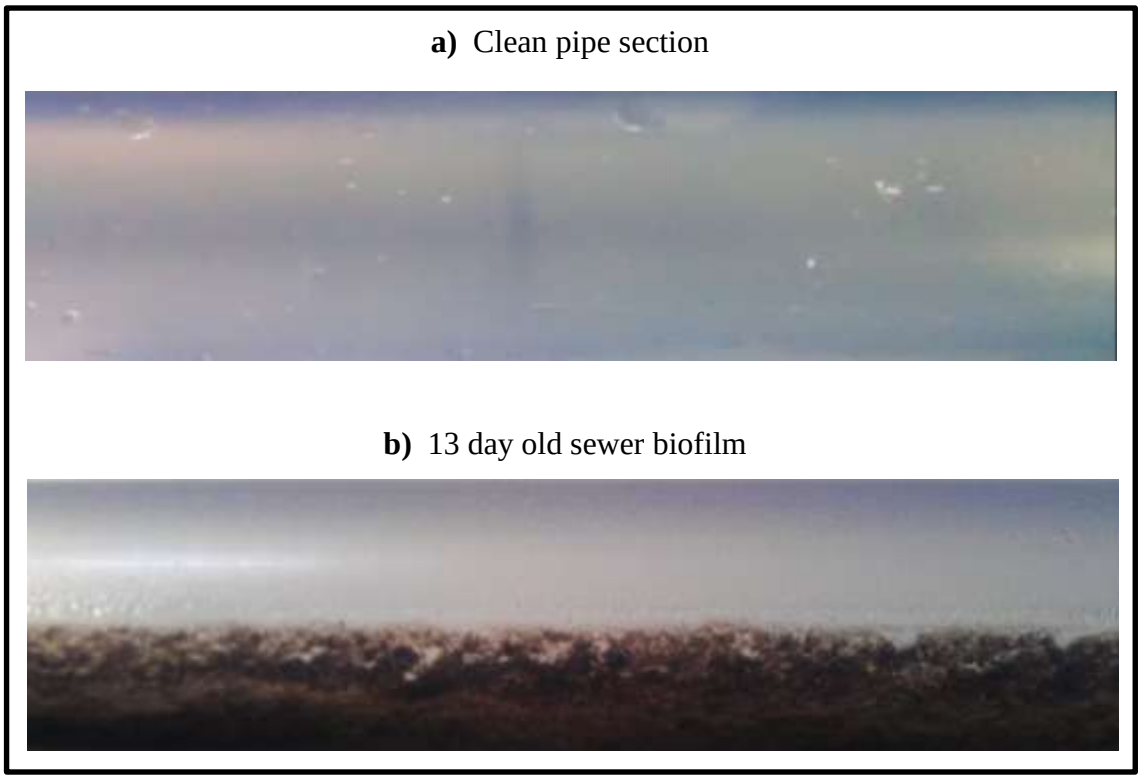


Figure 4.9 Sewer biofilm pipe section views for; a) clean biofilm pipe, b) 13 day old sewer biofilm.

The low N_2O emissions in this study highlights the potential issues with scaling up the pilot scale EWS to a real sewer environment. A possible explanation is there was inefficient substrate permeation into the biofilm. The HRT in the pilot scale sewer was short (14 seconds) with a flow rate of 1 L.min^{-1}, which could have resulted in periodic starvation of the biofilm, thus leaving some of the nitrifying population in dormant mode.

b) CFBBR system

As a possible solution, a sidestream biofilm reactor (CFBBR) for the N-Tox was developed, with the aim of sustaining a higher abundance of nitrifiers and providing longer residence times in a protected environment. A CFBBR was chosen instead of suspended or immobilised growth reactors due to washout of sludge at low HRT for the latter (Pan et al., 2004). Once a steady state was reached, baseline N_2O emissions from the CFBBR biofilm ranged between 7 ppm and 81 ppm, with an average of 22 ppm,

representing an order of magnitude higher than emissions from the sewer biofilm (see section 4.6.4).

A media fill fraction of 14 %v/v was identified to be the maximum possible before interruption of the flow. This was determined by running the reactor with increasing quantities of media, until the circulating flow stopped. This equated to an apparent solids hold up of 14 %v/v and a biofilm growth area of 0.5 m^2. Overall gas hold-up in the reactors was subsequently calculated as 0.319, resulting in a volumetric oxygen mass transfer coefficient of 754.6 hr^{-1}. The theoretical nitrogenous oxygen demand, nitrifier oxygen saturation constant and critical oxygen concentration were assumed to be 4.57 g-O_2.g-NH_4-N^{-1}, 1.3 g-O_2.m^{-3} and 4.0 g-O_2.m^{-3} respectively (Lazarova et al., 1997). With an average DO of 2.5 mg.L^{-1} during normal operation, the oxygen mass transfer rate was calculated as 4.8 kg-O_2.m^{-3}.hr^{-1} (Appendix 3). After introduction of a pre-grown biofilm to the CFBBR systems (i.e. post stage I – bacterial seed and stage II – biofilm pre-growth period), a 27-day acclimatisation period was observed between day 92 and 119 (Figure 4.10).

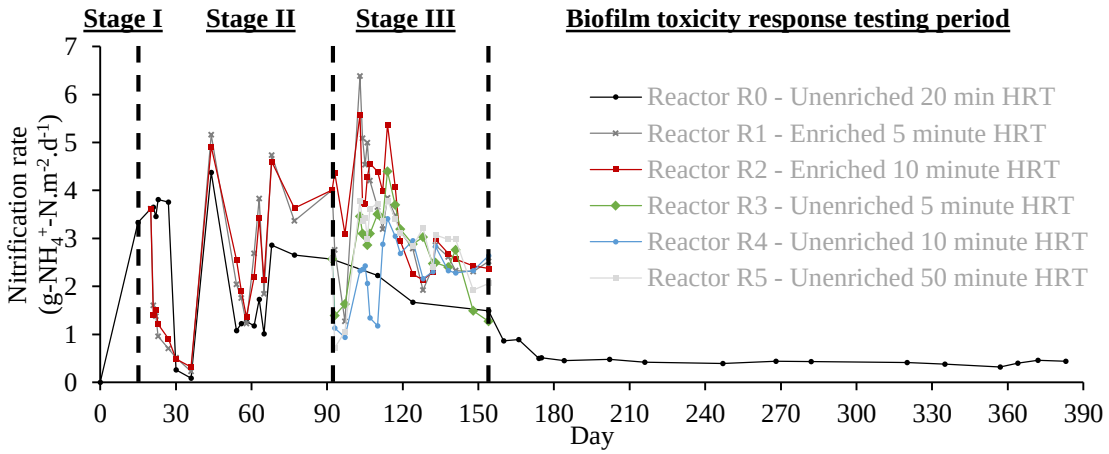


Figure 4.10 Nitrification performance of CFBBR biofilm at varying HRT, displaying; Stage I – 14 day bacterial seed period, Stage II – 78 day Biofilm pre-growth period, Stage III – 62 day HRT testing stage with a 27 day acclimatisation period. Post day 180 the steady state period was reached and all toxic response testing was undertaken after this point.

After the 27 day acclimatisation period in stage III, specific nitrification rates of around 2.5 g-NH_4^+-N.m^{-2}.d^{-1} were observed for all conditions (Figure 4.10). A 10 minute HRT utilising an unenriched biofilm had average nitrogen and organic loading rates of 150 g-NH_4^+-N.m^{-2}.d^{-1} and 1100 g-COD.m^{-2}.d^{-1}. Despite being 5.5 times larger than the maximum reported design organic loading rate of 200 g-COD.m^{-2}.d^{-1} for biofilm on

Kaldnes media (Rusten et al., 2000), nitrification performance was stable (Figure 4.10), suggesting no benefit to enriching the biofilm for nitrifiers.

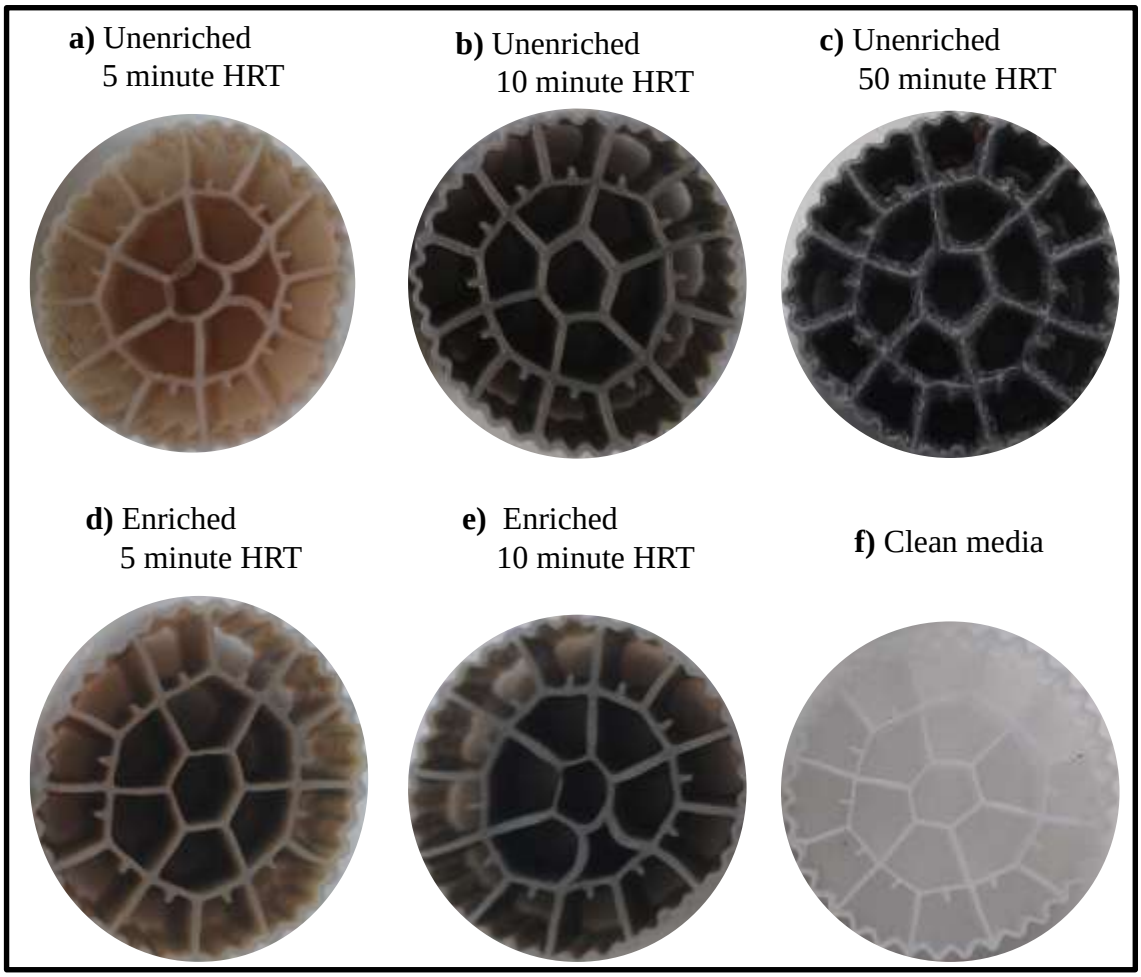


Figure 4.11 CFBBR reactor media plan views for; a) Unenriched 5 minute HRT biofilm, b) Unenriched 10 minute HRT biofilm, c) Unenriched 50 minute HRT biofilm, d) Enriched 5 minute HRT biofilm, e) Enriched 10 minute HRT biofilm, f) Clean Kaldnes K3 media.

After a period of 180 days post inoculation a true steady state was reached. The un-inhibited specific nitrification rates remained stable about 0.4 g-NH_4^+-N.m^{-2}.d^{-1} (Figure 4.10) with a standard deviation of 0.06. These rates remained stable throughout the biofilm toxicity response testing period (conducted at least 200 days post stage I inoculation).

4.6.4 Phase IV: Characterising biofilm responses to toxic shock

Biofilm community structure varies with respect to the environmental conditions it is exposed to and the binding surface (biotic or abiotic). The bacteria involved in EPS excretion and the formation of a heterogeneous matrix varies between different sessile

communities directly affecting the architecture of the biofilm. As such, the biofilm could form periphytons (on submerged solid surfaces), stromatolites (mushroom like structures predominantly found in quiescent waters) or streamers (filamentous biofilms predominantly found in flowing water) (Koechler et al., 2015).

Based on the above, the sewer biofilm in this study potentially exhibited streamer architecture, due to exposure to flowing water. Attachment surface characteristics, flow, level and velocity were fixed across the development period and the test stage to provide some level of control in biofilms architecture and responses to toxic events.

The CFBBR system aims to control biofilm architecture by fixing the attachment surface characteristics (using uniform floating biofilm carriers), flow rate, velocity and DO. However, even with these fixed characteristics, nutrient availability will vary depending on the sewage composition leading to different biofilm community structures. Hence, the CFBBR biofilm tested in this study was developed over a long time period in reactor R_0 to allow exposure and resilience to varying sewage compositions.

To allow swift toxicity detection, it was necessary to operate the CFBBR at low HRTs. Crude sewage typically contains high concentration of readily biodegradable BOD (Demirel et al., 2005) and this is compounded by the low HRT. These conditions can lead to proliferation of filamentous bacteria as they have a larger surface area than zoogleal bacteria, out-competing them for organic substrates and resulting in poor biofilm performance (Tchobanoglous et al., 2014d). An effective method for prevention of filamentous growth is to provide an initial anoxic contact zone (Tchobanoglous et al., 2014d). The non-aerated down-comer shaft of the CFBBR provided these conditions (Cui et al., 2008; Eldyasti et al., 2011; Lazarova and Manem, 1996; Li et al., 2012; Nogueira et al., 2002), thus promoting the selective growth of zoogleal organisms. Rapid uptake of soluble BOD in the down-comer leaves very little available for assimilation by filamentous organisms as well as providing conditions for biological denitrification (Tchobanoglous et al., 2014d).

The downside to a low HRT is a low nitrification rate, which may result in a biofilm with lower sensitivity to nitrification inhibitors than the secondary treatment process it is protecting. Thus, in this study the response of the biofilm to toxicity was rationalised

and compared to MLSS systems through dose response tests and toxic shock events. The size of the CFBBR reactor vessel was constrained by the size of the pumps available for bench scale testing, limiting the number of biofilm carriers. In practice, if the CFBBR system displays very low sensitivity to toxicity, the gaseous release can be amplified by:

- Maintaining the same headspace volume as the CFBBR in this study
- Increasing the number of biofilm carriers by increasing the volume of the CFBBR
- This will require a larger feed pump to maintain a 10 minute HRT

a) Nitrification Inhibition

The measured inhibitory effects of the four chemicals tested were significantly different from reported literature values and among the different reactors (Table 4.7). In general, the biological cultures tested in this work proved more resilient to toxic events than AS systems reported in the literature.

Table 4.7 Comparison of inhibitory effect (measured using dose response tests in this study, section 4.5.3) of known toxicants to the sewer biofilm, CFBBR reactor biofilm and MLSS. Reported literature values for MLSS are also displayed for comparison with the measured values.

Substance	Concentration ($mg.L^{-1}$)	Nitrification inhibition				
		Sewer biofilm pipes	CFBBR reactor	MLSS 10.5 $mg.L^{-1}$	MLSS 2850 $mg.L^{-1}$	Literature value (Hayes et al., 1998; Pagga et al., 2006)
ATU	0.5	76 %	35 %	33 %	34 %	50 %
	2.0	81 %	51 %	50 %	50 %	75 %
Copper (II)	1.5	46 %	76 %	5 %	-13 %*	50 %
	6.0	45 %	100 %	9 %	-7 %*	75 %
Chromium (VI)	16	35 %	30 %	50 %	14 %	50 %
	64	64 %	28 %	65 %	24 %	75 %
Nickel (II)	8.2	14 %	35 %	53 %	14 %	50 %
	33	42 %	68 %	90 %	16 %	75 %

*negative percentage indicates an increase in nitrification rate

The scale of inhibitory effect based on calculated EC_{50} concentrations (from the dose response analysis) of the tested toxicants was Cu^{2+} > ATU > Ni^{2+} > Cr^{6+} for the CFBBR reactor biofilm, ATU > Cu^{2+} > Ni^{2+} > Cr^{6+} for 2850 $mg.L^{-1}$ MLSS and ATU > Ni^{2+} >

Cr^{6+} > Cu^{2+} for the 10.5 mg.L^{-1} MLSS. For the sewer biofilm the scale of inhibitory effect based on measured nitrification inhibitions was ATU > Cu^{2+} > Cr^{6+} > Ni^{2+} (Table 4.7).

Biofilm systems have been reported to have a higher resilience to heavy metal toxicity than suspended growth cultures (Hayes et al., 1998; Lee et al., 2009; Weon et al., 2004) but less resilient to ATU (Hayes et al., 1998; Pagga et al., 2006) in relation to nitrification performance. This was shown to be the case in this study for ATU, nickel (II) and chromium (VI), but the biofilm systems were found to be less resilient to copper (II) toxicity than the suspended growth systems.

A 6 mg.L^{-1} copper (II) shock inhibited nitrification in 10.5 mg.L^{-1} and 2850 mg.L^{-1} MLSS systems by 9 % and -7 % respectively (Figure 4.13), with the latter indicating an increase in nitrification performance. Indeed, copper (II) concentrations up to ~45 mg.L^{-1} improved nitrification rate by up to 15 % in comparison to ~32 mg.L^{-1} in the literature (Barber and Stuckey, 2000; Cabrero et al., 1998; Lee et al., 2009; Ochoa-Herrera et al., 2011; Scullion et al., 2007), with a reduction in performance above this critical concentration.

From the dose response analysis, the copper (II) EC_{50} concentration was determined to be 0.42 mg.L^{-1} for the CFBBR biofilm, 52.0 mg.L^{-1} for a 10.5 mg.L^{-1} MLSS and 86.9 mg.L^{-1} for a 2850 mg.L^{-1} MLSS cultures (Figure 4.12). The EC_{50} values for suspended growth systems was higher than the reported range of 1.1 – 33 mg.L^{-1} (Beyenal et al., 1997; Gutiérrez et al., 2002; Hayes et al., 1998; Ochoa-Herrera et al., 2011; Weon et al., 2004). The response profile of the 3 systems was very different, with hill slope factors of 1.0, 3.7 and 4.5 for biofilm, 10.5 mg.L^{-1} MLSS and 2850 mg.L^{-1} MLSS respectively. Comparing the 3 curves using an F-test returned a p value of $<1 \times 10^{-4}$ and an r^2 of 0.30 resulted after attempting to fit the same model to all three datasets. Therefore, each system responds significantly differently to copper (II). For the sewer biofilm, 1.5 mg.L^{-1} copper (II) inhibited nitrification by 46 % in comparison to 76 % for the CFBBR reactor (Table 4.7), demonstrating a high sensitivity in comparison to a reported biofilm EC_{50} of 5.8 mg.L^{-1} (Weon et al., 2004).

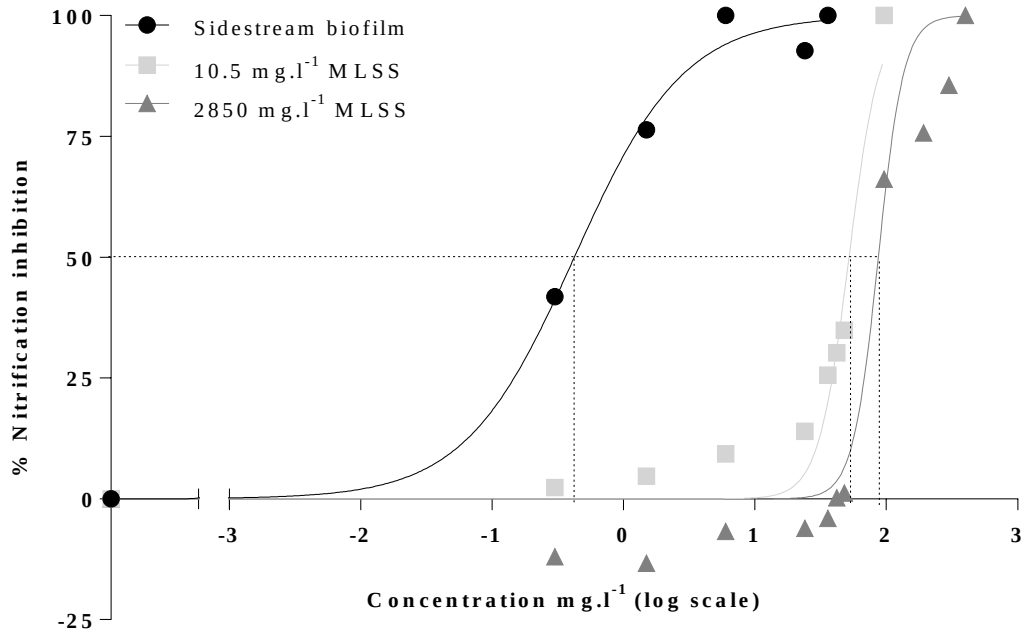


Figure 4.12 Copper (II) dose response curves for CFBBR sidestream reactor biofilm and MLSS (note y-axis intersects x-axis at -10). A dose response curve has been fitted to the data set, and the EC_{50} concentration has been determined.

Reported ATU EC_{50} values for suspended growth system ranged from 0.5 $mg.L^{-1}$ to 1.5 $mg.L^{-1}$ (Hayes et al., 1998; Pagga et al., 2006). The upper value in this range agrees with the measured ATU EC_{50} concentrations of 1.0 $mg.L^{-1}$, 1.3 $mg.L^{-1}$ and 1.7 $mg.L^{-1}$ for CFBBR biofilm, 10.5 $mg.L^{-1}$ MLSS and 2850 $mg.L^{-1}$ MLSS systems respectively in this study (Figure 4.13). Comparison of the response profiles again returned a low p value of 8×10^{-4} albeit higher than with copper (II), again suggesting the response profiles are significantly different. However, the hill slope factors were more comparable to each other at 0.9, 0.6 and 0.6 for biofilm, 10.5 $mg.L^{-1}$ MLSS and 2850 $mg.L^{-1}$ MLSS respectively. Indeed, when fitting the same model to each data set, an r^2 of 0.91 suggests a much more comparable response to ATU between the 3 systems. Lastly, the sewer biofilm did demonstrate lower resilience to toxicity than the other systems, with 0.5 $mg.L^{-1}$ ATU inhibiting nitrification by 76 % for the sewer biofilm in comparison to 35 % for the CFBBR biofilm (Table 4.7).

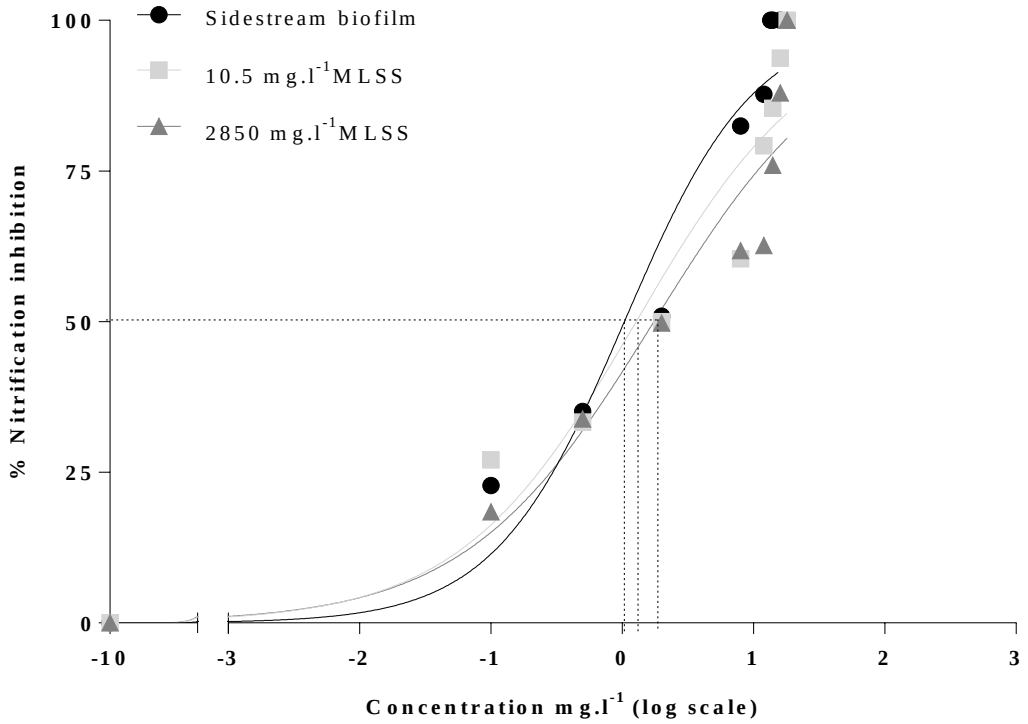


Figure 4.13 ATU dose response curves for CFBBR sidestream reactor biofilm and MLSS (note y-axis intersects x-axis at -10). A dose response curve has been fitted to the data set, and the EC_{50} concentration has been determined.

With Nickel (II), reported EC_{50} values for suspended growth systems ranged from 0.9 $mg.L^{-1}$ to 33 $mg.L^{-1}$ (Cokgor et al., 2007; Hayes et al., 1998; Weon et al., 2004). The measured EC_{50} concentration of 5.8 $mg.L^{-1}$ nickel (II) for 10.5 $mg.L^{-1}$ MLSS fits into this range, however, the 2850 $mg.L^{-1}$ MLSS system had an EC_{50} of 211.4 $mg.L^{-1}$ (Figure 4.14). The resilience of the CFBBR biofilm to nickel (II) was higher than the comparable floc system, with a measured EC_{50} of 13.0 $mg.L^{-1}$. It was also more resilient than the sewer biofilm, with the inhibitory effect of 8.2 $mg.L^{-1}$ nickel (II) at 42 % nitrification inhibition, in comparison to 35 % for the CFBBR biofilm (Table 4.7). Both biofilm systems showed lower sensitivity to nickel (II) in comparison to a reported biofilm EC_{50} of 2.9 $mg.L^{-1}$ (Weon et al., 2004).

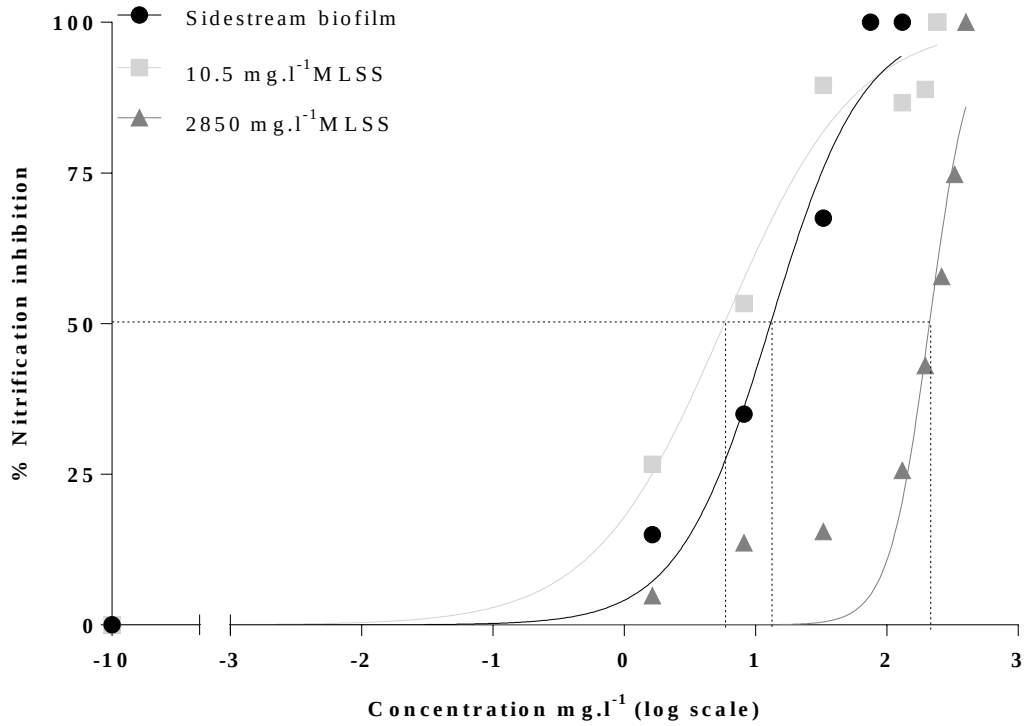


Figure 4.14 Nickel (II) dose response curves for CFBBR sidestream reactor biofilm and MLSS (note y-axis intersects x-axis at -10). A dose response curve has been fitted to the data set, and the EC_{50} concentration has been determined.

As with copper (II), a very low p value of $<1 \times 10^{-4}$ demonstrated significant difference between the nickel (II) response profiles, and indeed hill slope factors of 1.2, 0.9 and 2.8 for CFBBR biofilm, 10.5 $mg.L^{-1}$ MLSS and 2850 $mg.L^{-1}$ MLSS respectively agree with this. However, an r^2 of 0.63 showed stronger similarity between the responses than with copper (II).

In the literature, reported chromium (VI) EC_{50} values for suspended growth systems range from 16 $mg.L^{-1}$ to 60 $mg.L^{-1}$ (Cokgor et al., 2007; Gutiérrez et al., 2002), agreeing with the measured EC_{50} of 15.1 $mg.L^{-1}$ for 10.5 $mg.L^{-1}$ MLSS. As with nickel (II), the 2850 $mg.L^{-1}$ MLSS system's measured EC_{50} was over the reported range at 404 $mg.L^{-1}$ (Figure 4.15). The CFBBR biofilm was again more resilient than the comparable floc system, with the EC_{50} concentration determined to be 76.0 $mg.L^{-1}$. For the sewer biofilm, the inhibitory effect of 16 $mg.L^{-1}$ chromium (VI) was a 35 % drop in nitrification rate, similar to 30 % for the CFBBR biofilm (Table 4.7).

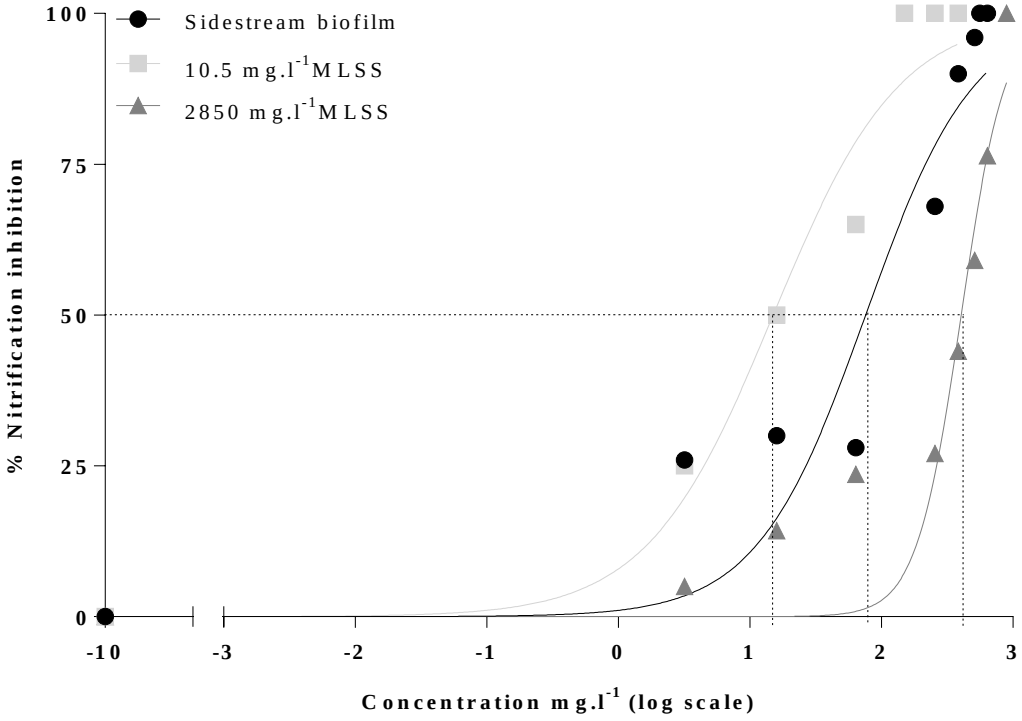


Figure 4.15 Chromium (VI) dose response curves for CFBBR sidestream reactor biofilm and MLSS (note y-axis intersects x-axis at -10). A dose response curve has been fitted to the data set, and the EC_{50} concentration has been determined.

The similarity between chromium (VI) response profiles was comparable to nickel (II), with an r^2 of 0.71. Hill slope factors were 1.0, 0.9 and 2.6 for CFBBR biofilm, 10.5 $mg.L^{-1}$ MLSS and 2850 $mg.L^{-1}$ MLSS respectively with a p value of $<1 \times 10^{-4}$. Hence, the response profiles of the three systems to chromium (VI) were very similar to nickel (II).

b) Biofilm off-gas responses to toxic events (Sewer and CFBBR)

Gaseous emissions for a typical 24 hour period (n = 9) have been recorded to analyse the standard deviation in emissions (Figure 4.16). The sewer biofilm N_2O emissions over a typical 24 hour period during normal conditions ranged between 0.4 – 12 ppm (Figure 4.16a). The CFBBR biofilm N_2O emissions over a 24 hour period displayed an emissions range of 6.8 – 85.2 ppm (Figure 4.16b). The CFBBR biofilm CO_2 emissions over a 24 hour period displayed an emissions range of 1018 to 1472 ppm (Figure 4.16c).

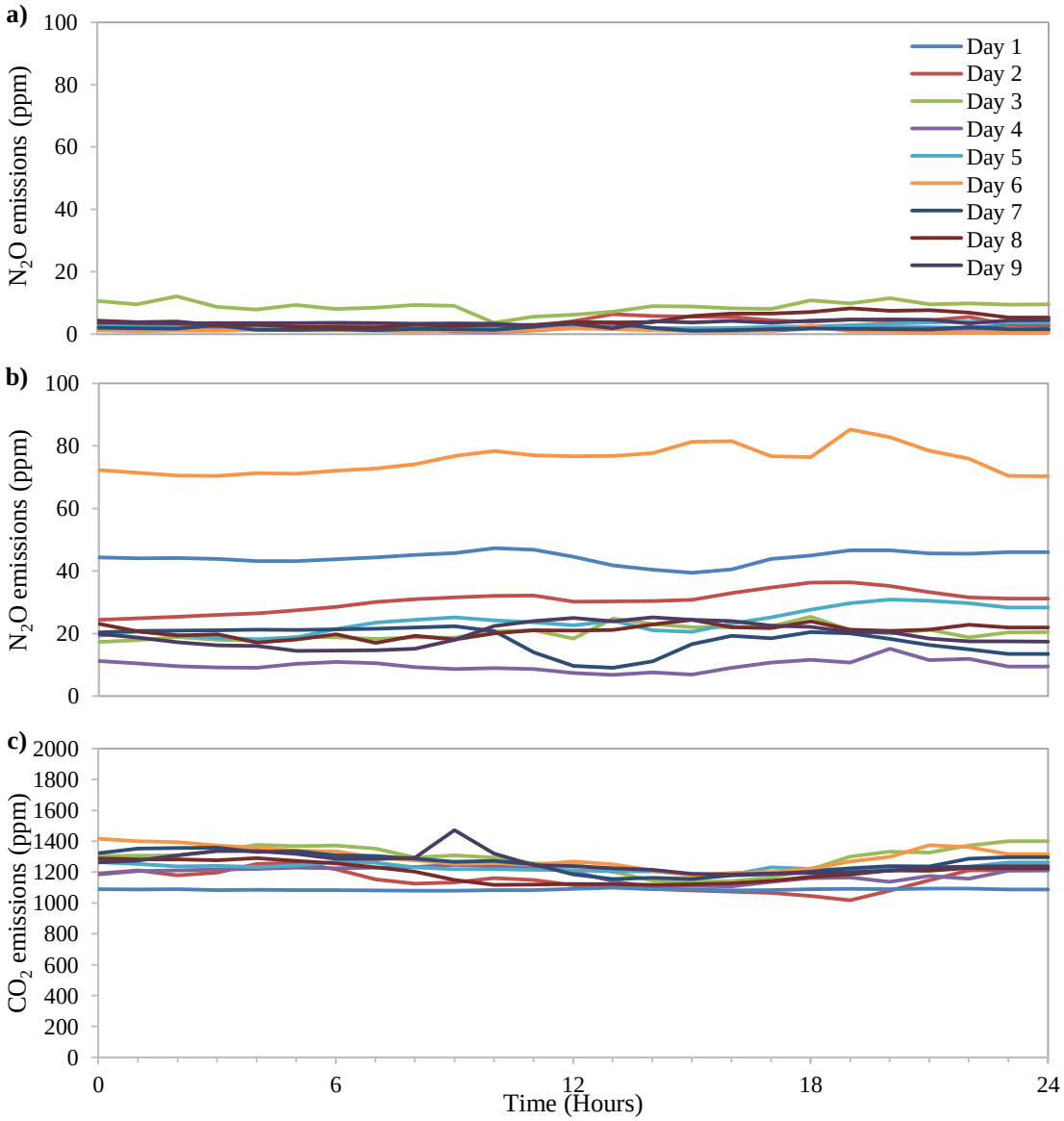


Figure 4.16 Hourly baseline emissions for 9 typical 24 hour periods (00:00 – 23:59) under un-inhibited conditions for; a) sewer biofilm N_2O; b) CFBBR N_2O; c) CFBBR CO_2**.**

All toxic shock event tests began between hour 13 and 14. The change in emissions over the shock period (90 minutes for sewer biofilm and 120 minutes for CFBBR biofilm) has been calculated for all toxicity tests and compared against the standard deviation in emissions during un-inhibited conditions (Table 4.8). The variation is discussed in the subsequent sections. Although pH was monitored in all toxicity tests, values remained stable in all cases, averaging pH 7.5, thus pH data is not discussed further.

Table 4.8 Change in emissions observed across the toxic shock period for the sewer biofilm and CFBBR systems. Change is compared against the standard deviation in emissions observed during un-inhibited conditions. Fields highlighted in green indicates the change is within the typical un-inhibited range, and red indicate change is over the typical un-inhibited range.

	Sewer biofilm	**CFBBR system**	
	N_2O, ppm	**N_2O, ppm**	**CO_2, ppm**
Un-inhibited conditions	2.6	19.0	87.5
ATU 0.5 mg.L^{-1}	2.6	-	-
ATU 2.0 mg.L^{-1}	2.2	-	-
ATU 6.5 mg.L^{-1}	-	6.0	184.1
ATU 16 mg.L^{-1}	-	2.3	80.4
ATU 32 mg.L^{-1}	-	3.3	15.0
Copper (II) 6 mg.L^{-1}	1.9	2.0	74.5
Copper (II) 24 mg.L^{-1}	-	12.1	219.0
Copper (II) 48 mg.L^{-1}	-	6.7	105.1
Copper (II) 96 mg.L^{-1}	-	16.3	599.7
Chromium (VI) 16 mg.L^{-1}	2.0	-	-
Chromium (VI) 64 mg.L^{-1}	0.6	-	-
Chromium (VI) 290 mg.L^{-1}		8.2	343.1
Nickel (II) 8 mg.L^{-1}	0.8	-	-
Nickel (II) 33 mg.L^{-1}	1.4	6.8	267.7
Nickel (II) 131 mg.L^{-1}	-	24.5	83.6

For the sewer biofilm post 0.5 mg.L^{-1} ATU shock, there was no significant change in N_2O emissions (Figure 4.17a). Emissions variation was within the recorded range (Table 4.8) under un-inhibited conditions, hence no significant response to 0.5 mg L^{-1} ATU was observed.

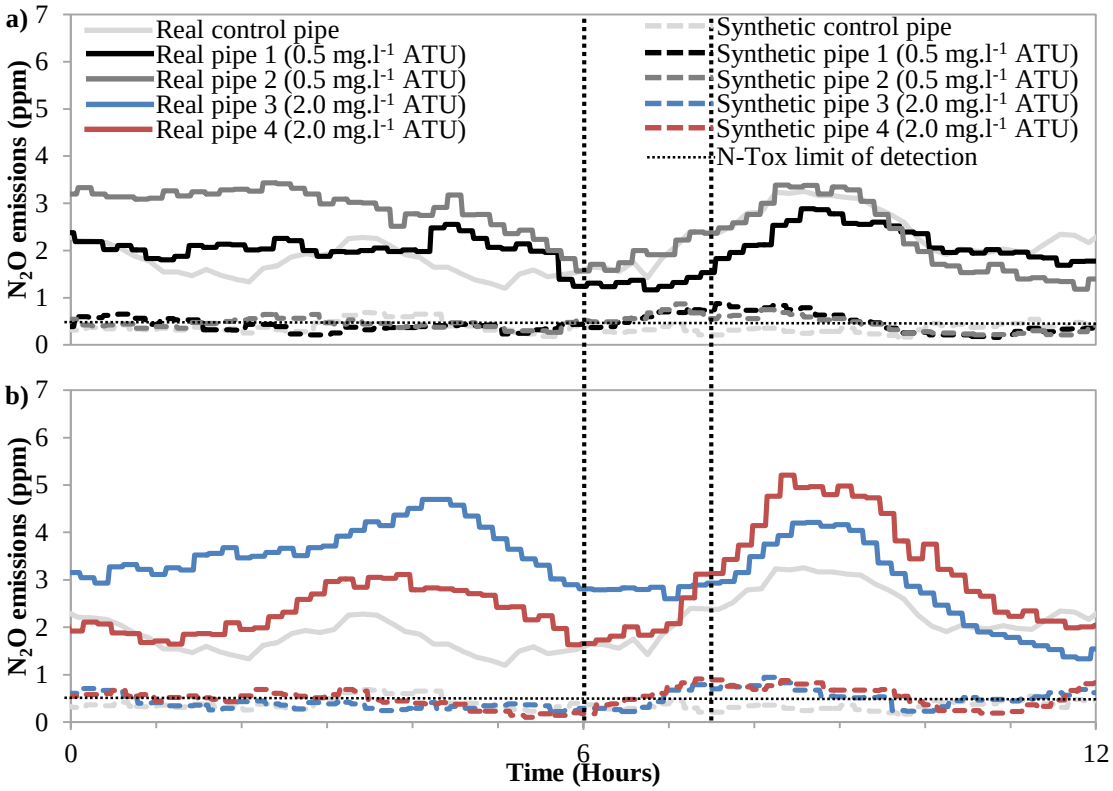


Figure 4.17 12 hour emissions profile for real and synthetically conditioned sewer biofilms. A 90 minute; a) 0.5 mg.L^{-1} ATU and b) 2 mg.L^{-1} ATU toxic shock was applied at 6 hours (within black dotted lines).

Post 2 mg.L^{-1}ATU shock, emissions of N_2O from the sewer biofilm conditioned with a real sewage feed did increase significantly ($p < 0.001$), comparing the emissions from inhibited to non-inhibited test results (Figure 4.17b). The control pipe also displayed an increase in emissions post shock (Figure 4.17b), attributed to variability in influent composition. This made it difficult to differentiate between a toxicity response and a natural peak. Similarly to the 0.5 mg.L^{-1} ATU shock, emissions variation was within the recorded range for un-inhibited conditions (Table 4.8).

As a comparison, the synthetically conditioned sewer biofilm displayed a much more stable baseline, with a 4 hour long N_2O peak (over and above the control baseline emissions) evident after the introduction of ATU (Figure 4.17). However, taking into account a measured 81 % inhibition to nitrification on addition of 2 mg.L^{-1} ATU (Table 4.7), the marginal change in emissions indicates little to no response. Furthermore, an N_2O emissions baseline below the limit of detection of 0.5 ppm (on average) for the synthetically conditioned biofilm indicates low nitrifier abundance in the sewer biofilm (Figure 4.17).

As with the sewer biofilm, the response of the CFBBR biofilm to ATU could not be characterised through N_2O, CO_2, or DO spikes during the shock duration. This was expected as ATU is a known inhibitor of ammonium oxidation in step 1 (Figure 1.1) with HDN and AH showing no sensitivity (Butler et al., 2009). As the only aerobic organisms affected were the autotrophs, and they likely occupied a small proportion of the biofilm, a hypoxic environment was not created, reflected in no DO spikes, and as such the NOS were not inhibited. Hence there was no pathway allowing N_2O accumulation and emission (Figure 1.1), reflected in no N_2O spikes (Figure 4.18a). With a 6.5 $mg.L^{-1}$ ATU shock, a steady decrease in CO_2 was observed between t = 6 and 8 hours (Figure 4.18a). However, in relation to the overall trend the decrease was marginal, indicated by a very shallow decline in CUSUM values (Figure 4.18b). The CO_2 emissions remained below the overall average of 1574 ppm for around 6.5 hours post shock (Figure 4.18b).

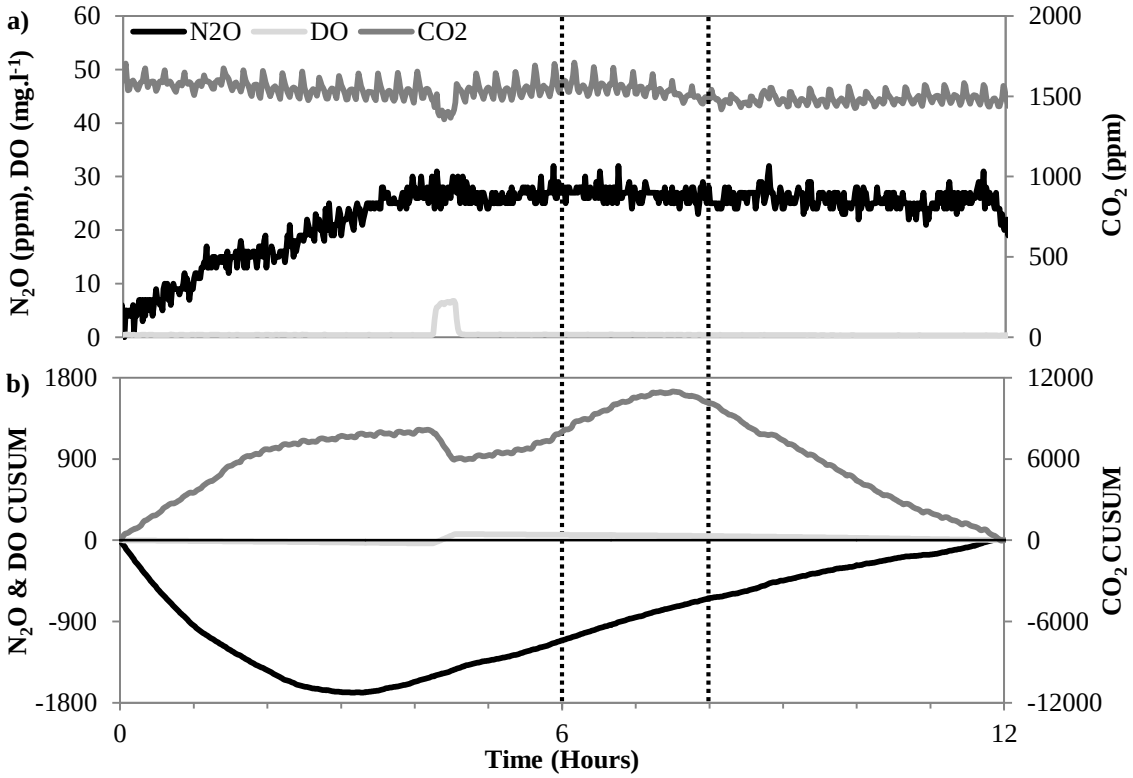


Figure 4.18 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) CUSUM control chart for change point detection. A 2 hour, 6.5 $mg.L^{-1}$ ATU shock was applied at 6 hours (within black dotted lines).

Emissions variation of CO_2 over the shock period was above the recorded range for un-inhibited conditions (Table 4.8). Emissions variation of N_2O was within the recorded range for un-inhibited conditions (Table 4.8).

A similar trend was also observed for 16 mg.L^{-1} and 32 mg.L^{-1} ATU shock tests, where in both cases a small negative change in CO_2 emissions occurred around 90 minutes after the start of the shock (Figure 4.19; Figure 4.20). A negative change in CO_2 CUSUM values was evident with emissions remaining below the overall average for 6 hours for 16 mg.L^{-1} ATU and 3 hours for 32 mg.L^{-1} ATU. Variation in N_2O and CO_2 emissions was within the recorded un-inhibited range (Table 4.8).

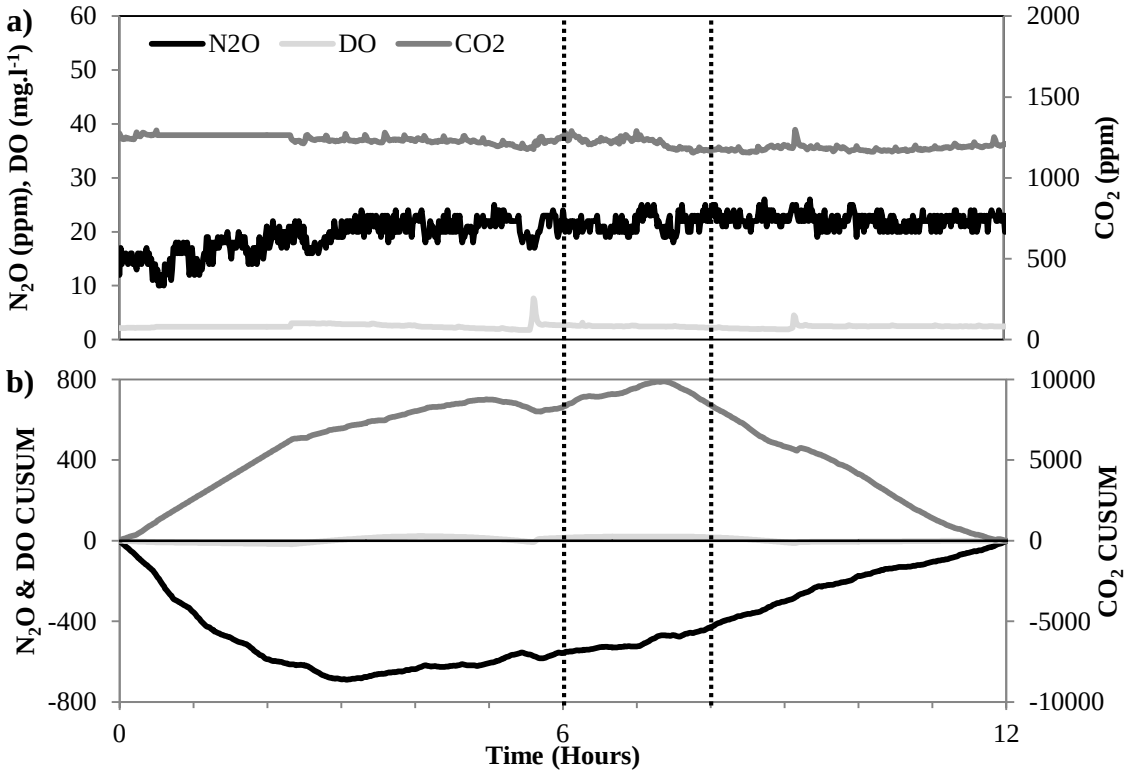


Figure 4.19 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 16 mg.L^{-1} ATU shock was applied at 6 hours (within black dotted lines).

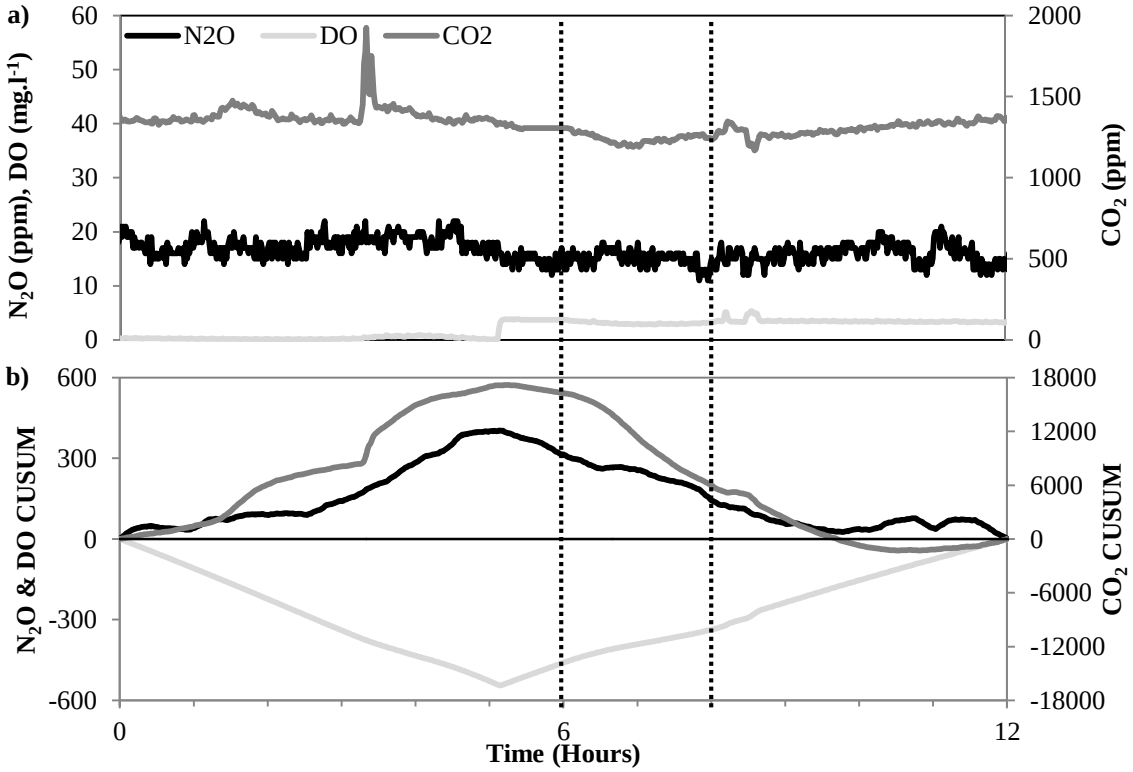


Figure 4.20 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 32 mg.L^{-1} ATU shock was applied at 6 hours (within black dotted lines).

Perhaps as a result of the low inhibitory effect of copper (II) on the sewer biofilm, there was no measurable N_2O response to a 6 mg.L^{-1} shock with comparable post shock emissions between the test and control pipes (Figure 4.21a). Any difference between the time series was most likely the result of natural variability between the biofilm in the two pipes, as influent composition was the same for the control and test pipes. Variation of N_2O emissions was within the reported un-inhibited range (Table 4.8).

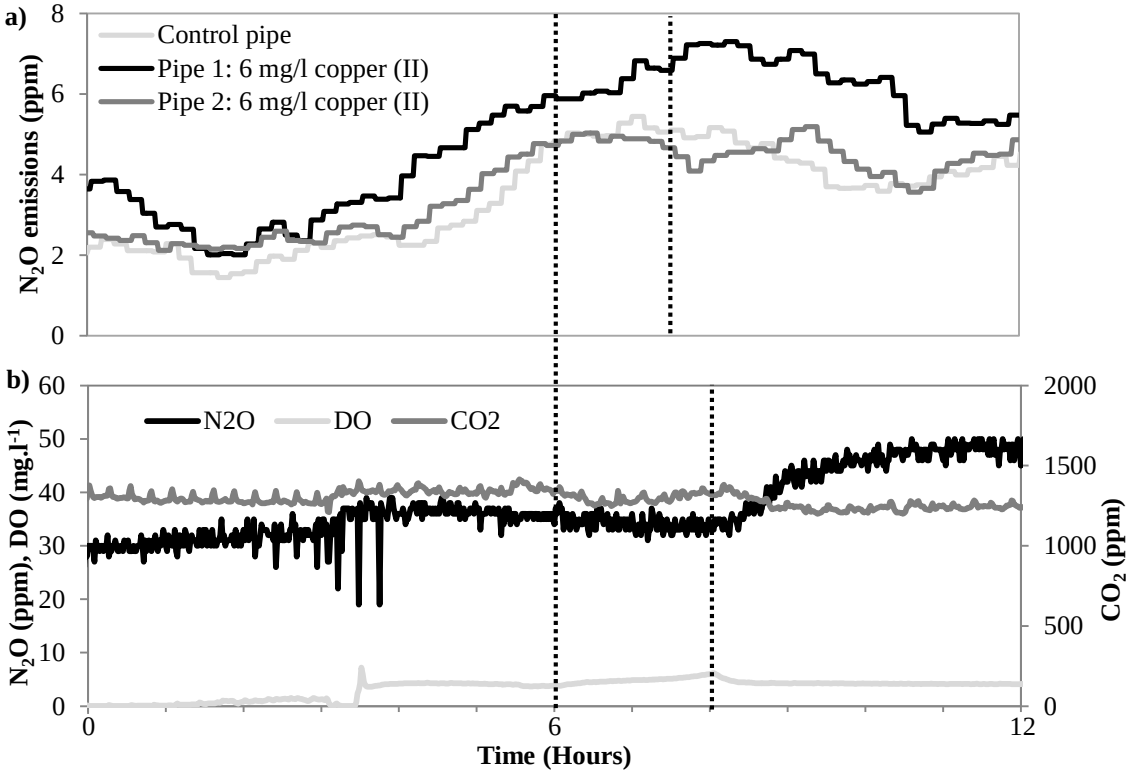


Figure 4.21 a) 12 hour emissions profile for a sewer biofilm; b) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm, A 1.5 hour and 2 hour 6 $mg.L^{-1}$ copper (II) shock was applied at 6 hours (within black dotted lines) for a and b respectively.

For the CFBBR biofilm, there was generally a positive correlation between toxicant concentration and gaseous emissions peak height. The lowest concentration tested, 6 $mg.L^{-1}$ copper (II), showed marginal changes across the shock duration. A measurable increase in DO was observed, 1.8 $mg.L^{-1}$ above the baseline, indicating a decrease in microbial respiration rate (Figure 4.21b). The significant change in DO at t = 3.5 hours was attributed to cleaning of the DO probe.

Indeed, a post shock response was potentially observed with a negative change in CO_2 and a rapid increase in N_2O emissions at t = 8 hours. However, the variation in CO_2 and N_2O emissions was well within the recorded un-inhibited range (Table 4.8).

Increasing the concentration of copper (II) to 24 $mg.L^{-1}$ displayed a measurable CO_2 spike and sharp CUSUM slope change of 100, lasting throughout the shock duration (Figure 4.22a; Table 4.9), potentially indicating inhibition to NOB (Oguz et al., 2006) or methanogen metabolism (Capone et al., 1983; Sanchez et al., 1996). A measurable increase in DO of 3.04 $mg.L^{-1}$ above the baseline was also recorded (1.7 times larger than with 6 $mg.L^{-1}$ Cu^{2+}) agreeing well with aerobic micro-organism inhibition (Figure

1.1). Indeed, the CO_2 emissions variation across the shock period was above the reported range for un-inhibited conditions but N_2O variation was within (Table 4.8). Additionally, the CUSUM data did suggest a response was observed.

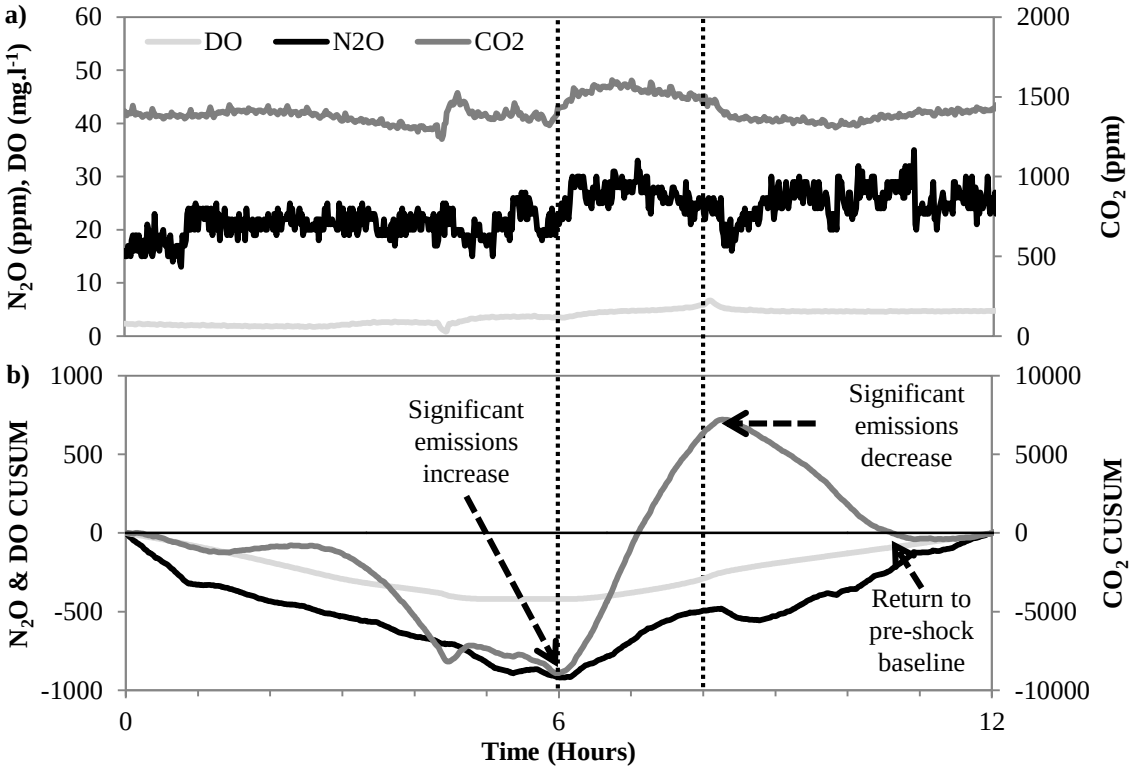


Figure 4.22 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 24 $mg.L^{-1}$ copper (II) shock was applied at 6 hours (within black dotted lines).

Table 4.9 CO_2 and N_2O CUSUM slope change points following a copper (II) shock.

		Copper (II) concentration, $mg.L^{-1}$			
		24	48	96	96 synthetic
Response to shock	CO_2 change point time , h	6.00	6.10	6.08	6.28
	CO_2 CUSUM Slope change	99.61	44.74	390.22	38.91
	N_2O change point time , h	6.15	6.17	6.08	6.12
	N_2O CUSUM Slope change	5.90	1.20	16.00	6.30
End of response	CO_2 change point time , h	8.23	8.22	8.12	8.52
	CO_2 CUSUM Slope change	-66.48	-58.41	-244.70	-72.11
	N_2O change point time , h	8.25	8.35	8.08	8.02
	N_2O CUSUM Slope change	-5.30	-1.40	-10.90	-7.40

The response was evident by an N_2O spike, where CUSUM data suddenly increased after t = 6 hours with a slope change of 5.90 (Table 4.9) and then almost flat lined towards the end of the 120 minute shock period. A post shock change was measured 20 minutes post shock, where the CO_2 emissions declined. Data remained below the

overall average, until t = 10.5 hours, where a return to pre-shock baseline was observed, suggesting a recovery period of 150 minutes.

However, the N_2O and CO_2 response was more prominent for a 24 $mg.L^{-1}$ copper (II) shock than 48 $mg.L^{-1}$ copper (II) despite the two fold increase in copper (II) concentration. As for the 24 $mg.L^{-1}$ copper (II) shock, the variation in CO_2 and N_2O emissions across the shock period were above and within the reported range for un-inhibited conditions respectively (Table 4.8). Smaller emissions peaks were observed with 48 $mg.L^{-1}$ copper (II) (Figure 4.23a) along with a shallow CUSUM slope of 45 and 1 for CO_2 and N_2O respectively (Figure 4.23b; Table 4.9). The increased inhibitory effect was evident in the DO response, with a sudden steep CUSUM incline over the shock (Figure 4.23b). Indeed, the end point (where the gradient of the DO CUSUM becomes shallower) was reached 33 minutes sooner with a 48 $mg.L^{-1}$ copper (II) shock in comparison to t = 7.5 hours for a 24 $mg.L^{-1}$ copper (II) shock load (Figure 4.23b). The DO max was reached at t = 8 hours (as with 24 $mg.L^{-1}$ copper) and was 6.55 $mg.L^{-1}$ above the baseline. This was 2 times higher than with 24 $mg.L^{-1}$ copper (II), correlating well with the two fold increase in concentration.

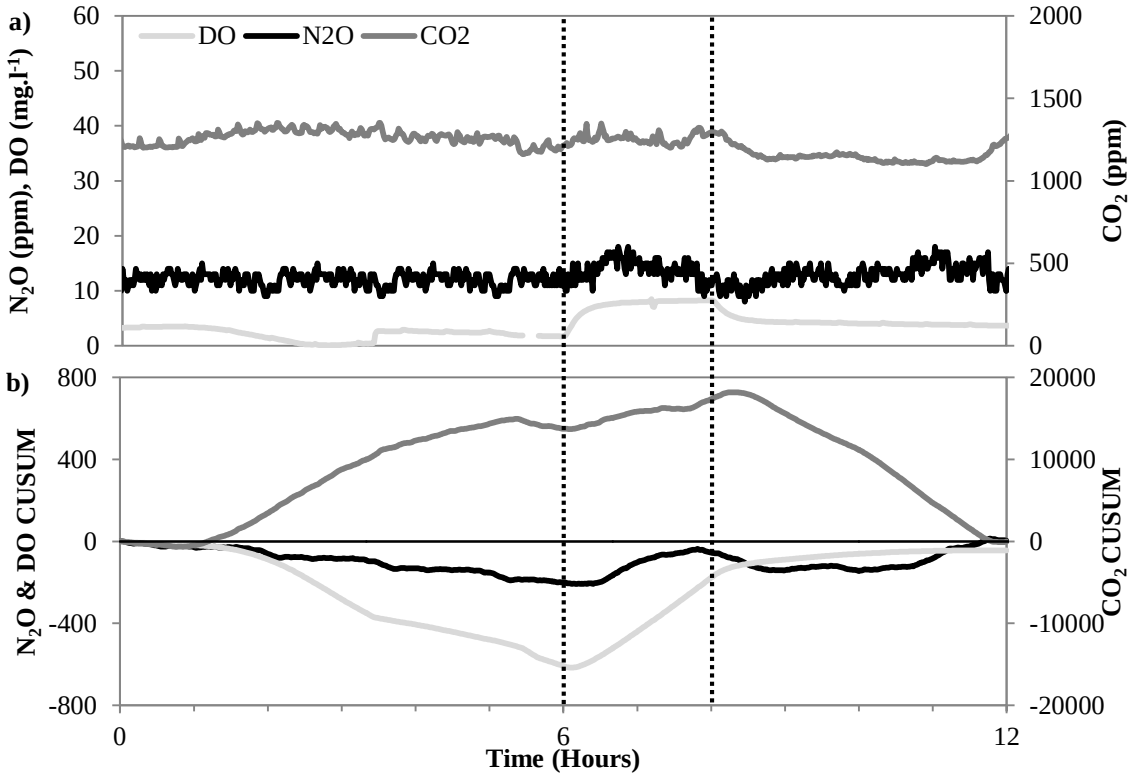


Figure 4.23 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 48 $mg.L^{-1}$ copper (II) shock was applied at 6 hours (within black dotted lines).

Increasing the copper (II) concentration two fold to 96 $mg.L^{-1}$ resulted in a rapid and large response in CO_2 and N_2O emissions (Figure 4.24a), represented with a steep CUSUM slope change of 390.22 and 16.00 for CO_2 and N_2O respectively (Figure 4.24b; Table 4.9). Variation for CO_2 was well above the reported range for un-inhibited conditions but N_2O variation was within the un-inhibited range (Table 4.8). The DO response was also rapid, with the end point reached 38 and 111 minutes sooner than 48 $mg.L^{-1}$ and 24 $mg.L^{-1}$ copper (II) shock loads respectively.

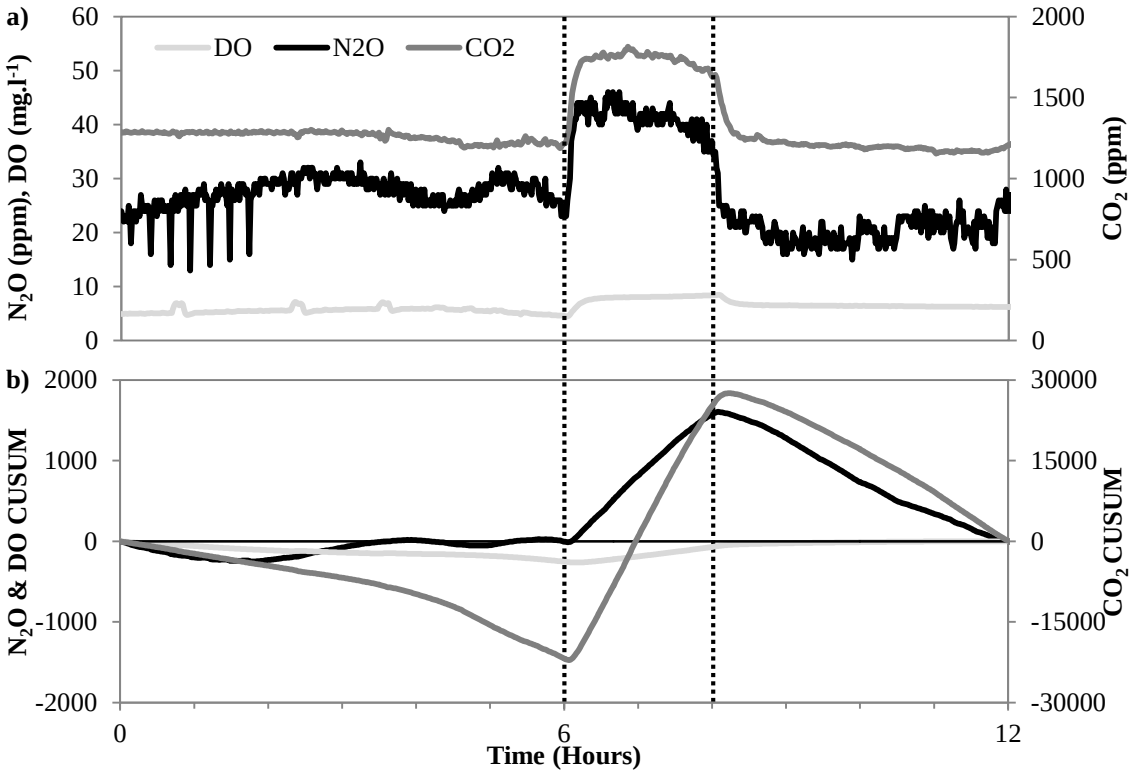


Figure 4.24 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 96 mg.L^{-1} copper (II) shock was applied at 6 hours (within black dotted lines).

In the settled sewage tests, large variations in emissions during an un-inhibited state were evident. Conducting tests with synthetic sewage allowed some variation to be removed. In general, the N_2O and CO_2 emissions data (Figure 4.25a) appeared more stable than with a settled sewage feed, with the latter displaying sporadic localised variation across the entire time series (Figure 4.24a). However, responses to toxicity appeared weaker with synthetic sewage. The CO_2 response was particularly weak, with CUSUM data failing to confirm significant changes over the shock period. The N_2O response was lower than with the settled sewage 96 mg.L^{-1} copper (II) test, but relatively high when compared to the 24 mg.L^{-1} and 48 mg.L^{-1} copper (II) tests. The spike was evident through a steep CUSUM slope change of 6.30, lasting throughout the shock period (Figure 4.25b; Table 4.9) and a sharp peak in emissions. Finally, the synthetic sewage solution had a high DO concentration close to the saturation concentration (calculated to be 8.75 mg-O_2.l^{-1} using Henry's law), meaning any increase would be marginal and so was removed from the plot.

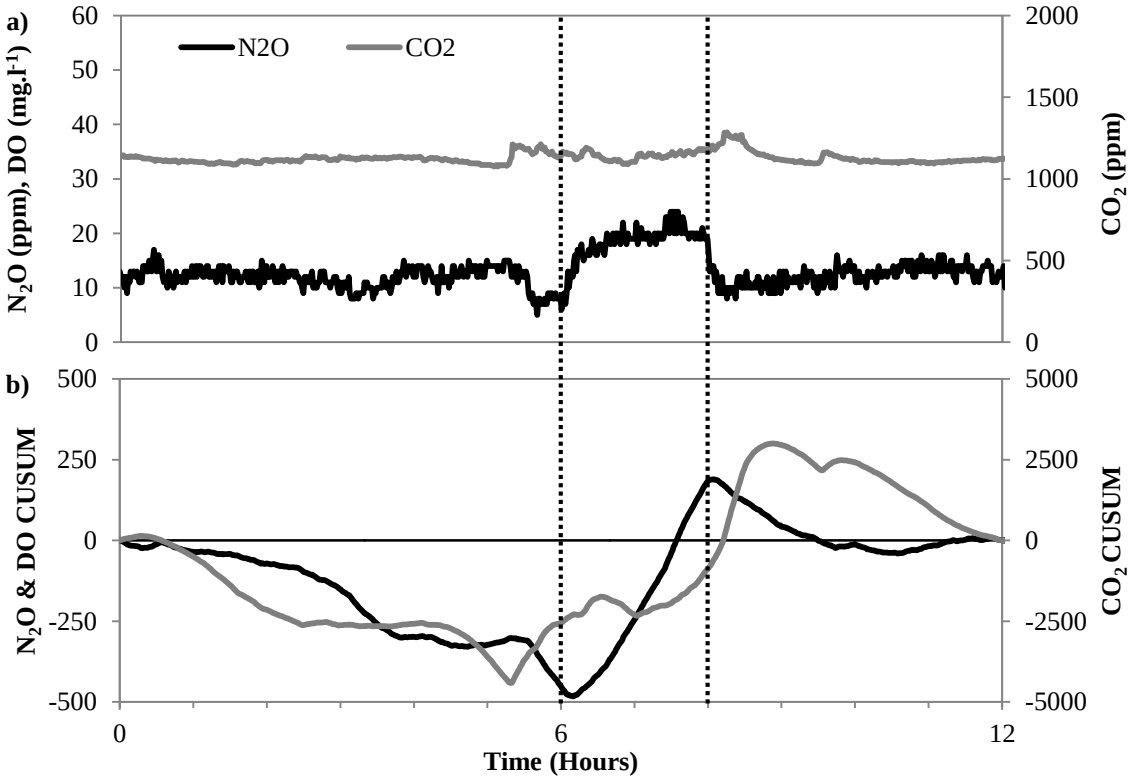


Figure 4.25 a) 12 hour CO_2 and N_2O profile for a synthetically conditioned CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 96 $mg.L^{-1}$ copper (II) shock was applied at 6 hours (within black dotted lines).

The response of the sewer biofilm to chromium (VI) and nickel (II) could not be characterised through N_2O emissions (Appendix 4; Appendix 5). Variation of emissions across the full period was within the recorded range for un-inhibited conditions (Table 4.8). There was no significant statistical difference between the control and test pipe emissions, or between pre and post shock emissions.

On the contrary, the CFBBR reactor displayed a clear N_2O response to 290 $mg.L^{-1}$ chromium (VI) (Figure 4.26a), despite emissions variation for the shock period being within the reported range for un-inhibited conditions (Table 4.8). A rapid jump to 41 ppm N_2O emissions was observed and confirmed by a 4.13 CUSUM slope change 10 minutes into the shock duration, one HRT after the shock was applied (Figure 4.26a; Table 4.10). The N_2O emission remained high before a steep decline 80 minutes into the shock duration occurred, evidenced by a -3 CUSUM slope change (Figure 4.26a; Table 4.10). By t = 8 hours, N_2O emissions had returned to the pre-shock baseline, and the change points were confirmed by the CUSUM data (Figure 4.26b).

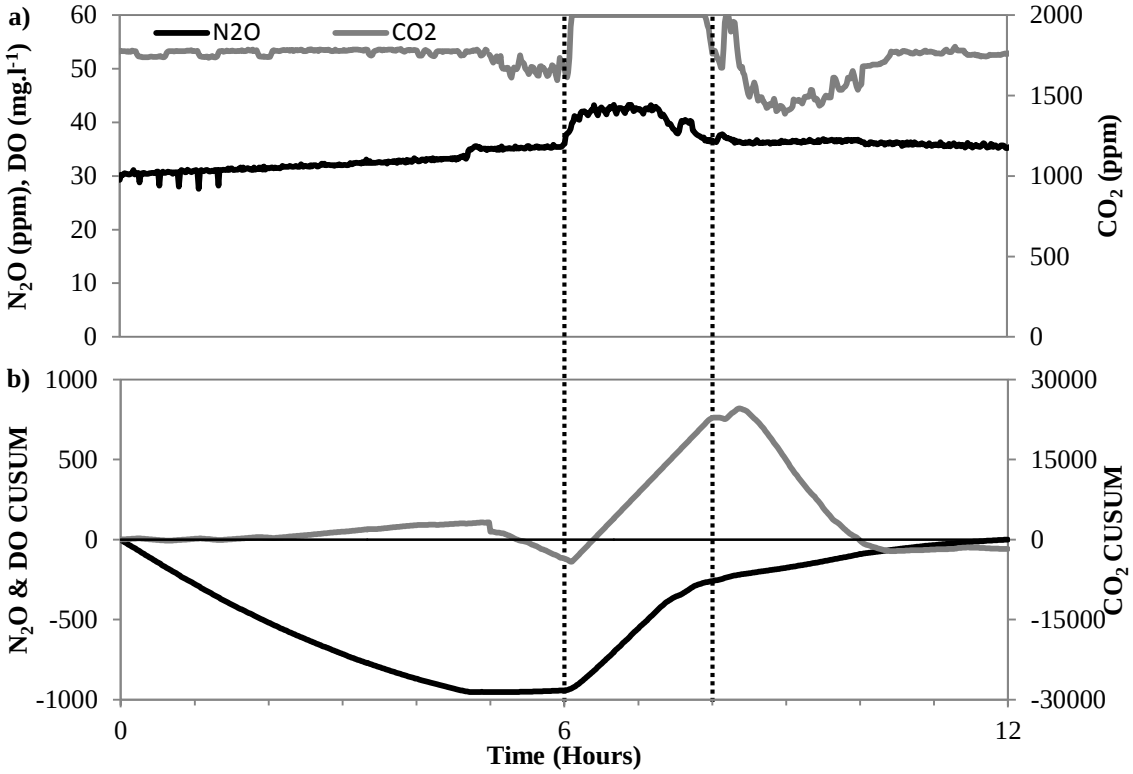


Figure 4.26 a) 12 hour CO_2 and N_2O profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 290 $mg.L^{-1}$ chromium (VI) shock was applied at 6 hours (within black dotted lines).

Table 4.10 CO_2 and N_2O CUSUM slope change points following chromium (VI) and nickel (II) shocks.

		Chromium (VI) concentration, $mg.L^{-1}$	Nickel (II) concentration, $mg.L^{-1}$	
		290	33	131
Response to shock	CO_2 change point time , h	6.1	6.7	6.1
	CO_2 CUSUM Slope change	318	97	50
	N_2O change point time , h	6.1	6.1	6.2
	N_2O CUSUM Slope change	4	4	8
End of response	CO_2 change point time , h	8.0	8.0	7.9
	CO_2 CUSUM Slope change	-231	-118	-79
	N_2O change point time , h	7.4	7.7	8.1
	N_2O CUSUM Slope change	-3	-2	-6

A very rapid change in CO_2 emissions was observed 10 minutes into the shock duration (Figure 4.26a), with a peak exceeding the range of the monitor (0 – 2000 ppm). The spike was evidenced by a steep change in CUSUM slope of 318 (Table 4.10), large enough to exhibit a lasting change in emissions until the shock was terminated. At this point, a steep decline in emissions was experienced (Figure 4.26a), evidenced by a sharp change in CUSUM slope of -231 (Figure 4.26b; Table 4.10). Post shock data remained

below the overall average, until t = 10 hours and 10 minutes, where a return to pre-shock baseline was observed, suggesting a recovery period of 130 minutes. The clear CO_2 response was supported by emissions variation well above the range recorded for un-inhibited conditions (Table 4.8).

Despite the clear gaseous response to a 290 $mg.L^{-1}$ chromium (VI) shock, a very marginal change in DO was observed across the shock duration, but the magnitude was not great enough to confirm the change point in CUSUM data. As such, DO was removed from the time series.

As with chromium (VI), a clear N_2O response to 33 $mg.L^{-1}$ nickel (II) was evident for the CFBBR biofilm (Figure 4.27a). Despite emissions variation being within the range for un-inhibited conditions (Table 4.8), emissions of N_2O increased rapidly to 55 ppm, 10 minutes into the shock duration (Figure 4.27a). The low variation may be a result of the actual response peak being very narrow. This change point was confirmed by a sharp CUSUM slope change of 4.20 (Figure 4.27b; Table 4.10), however, the 5 ppm increase above pre shock emissions, coupled with the steady decline to 42 ppm, 10 minutes after the shock was terminated, was not large enough to give a lasting effect. The CUSUM data suggests that around 90 minutes after the shock began a -2.10 slope change occurred, where N_2O emissions briefly flat lined about the overall average of 46.6 ppm (Figure 4.27b; Table 4.10), before dropping further. The emissions remained low for around 4 hours, before returning to a baseline above the overall average, suggesting a recovery period.

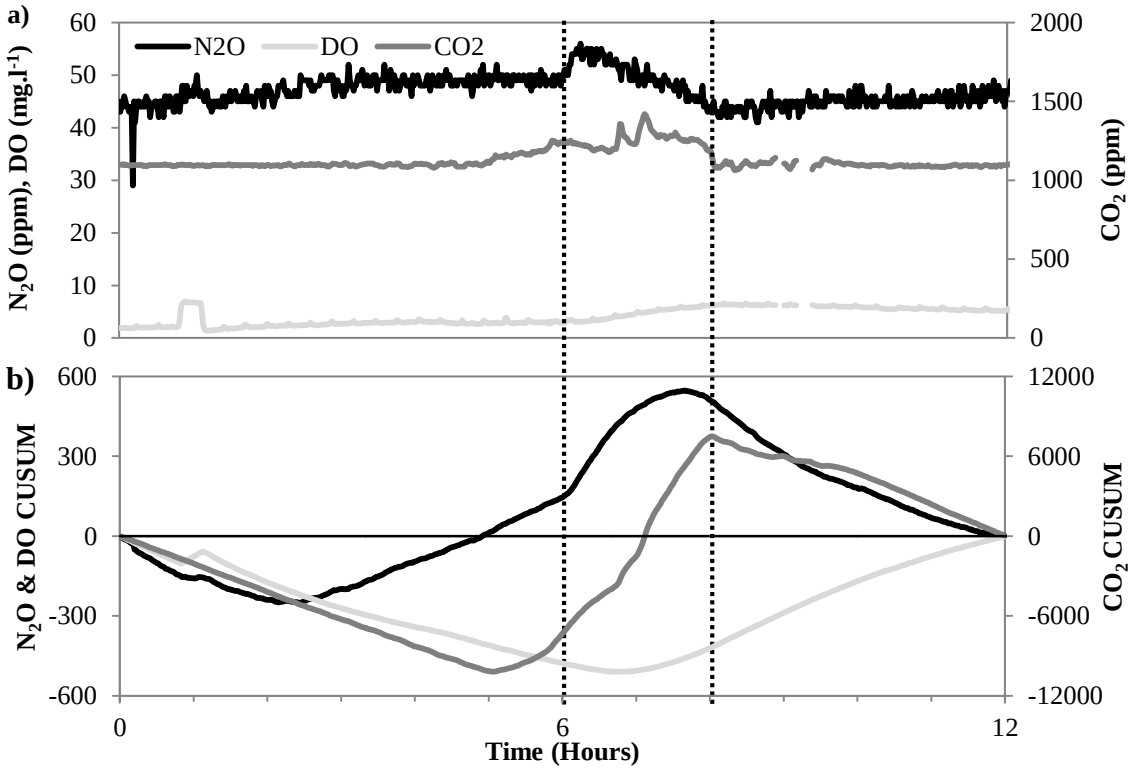


Figure 4.27 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 33 mg.L^{-1} nickel (II) shock was applied at 6 hours (within black dotted lines).

A rapid increase in CO_2 emissions was observed 44 minutes into the 33 mg.L^{-1} nickel (II) shock (Figure 4.27a) and emissions variation was above the reported range for un-inhibited conditions (Table 4.8). The spike was confirmed by a 97 CUSUM slope change (Table 4.10) and was large enough to exhibit a lasting change in emissions until the shock was terminated. At this point, a steep decline in emissions was experienced and evidenced by a -118 CUSUM slope change (Figure 4.27a; Table 4.10). The DO response was clear, with a steady rise post shock, indicating inhibition to aerobic microbial respiration (Figure 4.27a).

Increasing the concentration to 131 mg.L^{-1} nickel (II) displayed a rapid spike in N_2O emissions around 10 minutes after introduction of the shock (Figure 4.28a), represented by a steep CUSUM slope change of 8 (Figure 4.28b; Table 4.10). This was also evidenced by a high emissions variation well above the range for un-inhibited conditions (Table 4.8). Emissions stayed high until the shock was removed, and then rather than rapidly declining (as seen previously), emissions steadily declined over a 14 hour period (Figure 4.28a). No significant change in CO_2 emissions was observed at the start of the shock, evidenced by only a moderate CUSUM slope change of 50 (Table

4.10), at 10 minutes into the shock duration, potentially indicating inhibition to AH and HDN. A major change in CO_2 emission came 116 minutes into the shock duration, where a steep decline in emissions and a -79 CUSUM slope change was observed (Figure 4.28a). After this point, emissions remained low for around 6 hours, before returning to the baseline, suggesting a recovery period. However, the CO_2 emissions variation over the shock period was just within the range for un-inhibited conditions (Table 4.8).

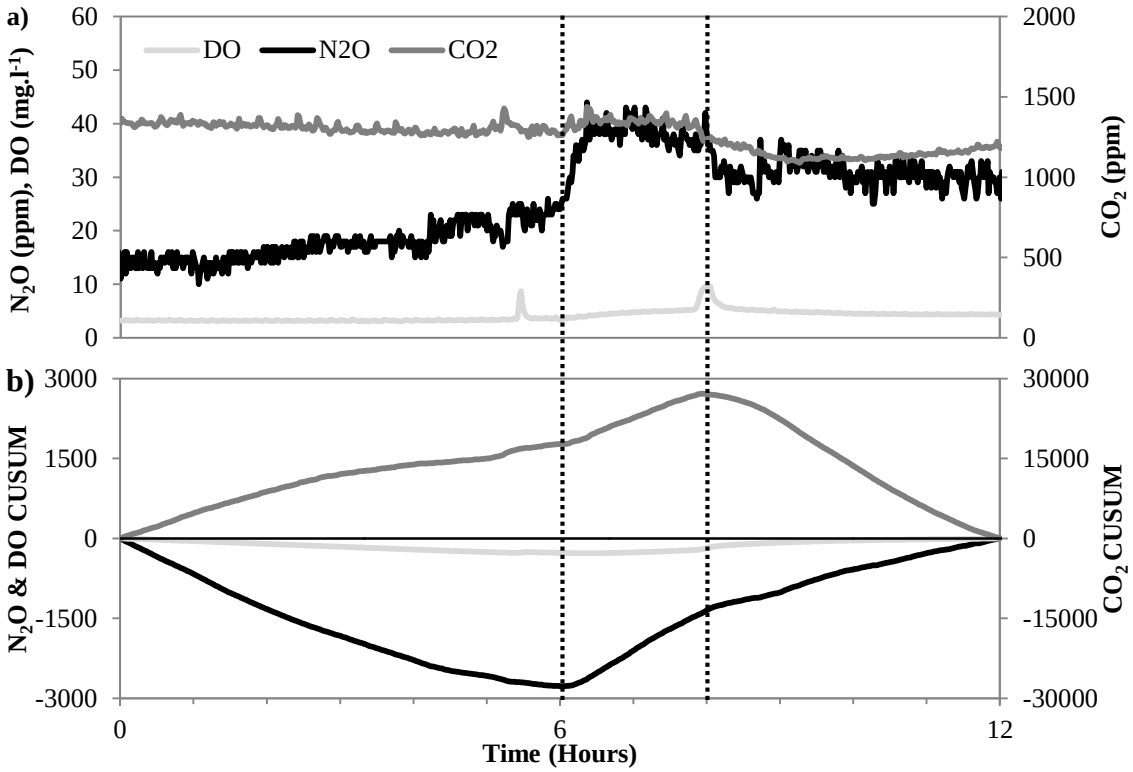


Figure 4.28 a) 12 hour CO_2, N_2O and DO profile for a CFBBR biofilm; b) Cumulative sum (CUSUM) control chart for change point detection. A 2 hour, 131 mg.L^{-1} nickel (II) shock was applied at 6 hours (within black dotted lines).

The DO response displayed a steady increase up to 110 minutes into the shock duration after which a sharp spike was observed, followed by a steady drop and a flat line. Interestingly, the DO concentration never fully returns to pre-shock concentrations (remaining on average 1 mg.L^{-1} higher post shock), perhaps indicating a lasting biocidal effect of nickel (II) at 131 mg.L^{-1}.

4.7 Discussion

This study aimed to identify a suitable on-line toxicity monitor, for use in an in-sewer EWS. Two monitoring techniques were identified as being potentially suitable, namely the Nitritox (liquid phase analysis) and N-Tox (headspace gas analysis) monitors. The performance of both techniques was tested in terms of sensitivity and response to N_2O concentration and the operational and maintenance requirements. To better understand the response to known nitrification inhibitors (ATU and heavy metals), biofilms and suspended growth cultures were tested in a CFBBR aimed at sustaining a sufficient abundance of nitrifiers at high organic loading rates and low HRT and to generate a gas response. Resilience to long-term operation with settled sewage at high organic and hydraulic loading rates was demonstrated along with clear responses to heavy metal toxicants in the CFBBR, while the sewer biofilm displayed no response.

The three main contributions to knowledge from this study are:

1) A nitrifying population can be established in the sewer pipe wall biofilm with a growth time of 13 days. When growing biofilm in an organic-rich feed at a minimum of 10 minutes HRT, the nitrifying community reaches a steady state at 190 days.
2) Under similar nitrification inhibition testing conditions, biofilms have a comparable sensitivity to ATU shocks than the equivalent suspended growth biomass. For heavy metals, biofilms are more sensitive to shocks with copper (II) but less sensitive to chromium (VI) and nickel (II).
3) The response of mixed culture biofilms to heavy metal toxicity can be detected through gaseous N_2O and CO_2 emissions profiling.

These findings are discussed in relation to the theoretical model proposed at the start of the study (Figure 1.1), and have been used as the basis for the design of an EWS for heavy metal toxicity detection in a sewerage network (CHAPTER 5).

4.7.1 Biofilm development

A nitrifying biofilm was established under high organic load and low HRT in sewer pipes and carrier media. This was evident in the ammonium removal rates observed in off-line assays (section 4.6.3). Whilst HRT between 10 and 50 minutes had no impact on long term nitrification rate of carrier media biofilm, operating the biofilm systems at 5 minutes HRT resulted in impaired long term performance. This suggests a threshold exists whereby the operating conditions become too stressful for the nitrifiers and are slowly outcompeted by other species.

Biofilm growth cycles are limited by their ability to successfully transfer oxygen and substrate from the bulk liquid into the biofilm, i.e. diffusion limited (Baban and Talinli, 2009; Tchobanoglous et al., 2014a). This is believed to limit autotrophic nitrification activity as DO is required for NH_4^+ and NO_2^- oxidation (Rostron et al., 2001). This is compounded when high organic loads are applied because typical biomass yield is 0.45 g-VS.g-substrate^{-1} for aerobic heterotrophs, higher than 0.12 g-VS.g-substrate^{-1} for AOB (Tchobanoglous et al., 2014c), leading to a lower AOB biomass fraction. Indeed, the low HRT's applied in this study resulted in high organic loading rates, leading to high competion for oxygen as an electron acceptor, which is potentially detrimental to nitrifying performance (Bassin et al., 2012).

Nitrifying activity in the CFBBR was lower than reported specific rates of 1.1 – 1.5 g-NH_4^+-N.m^{-2}.d^{-1} for moving bed biofilm reactor (MBBR) systems operated within design organic loading rates (Hem et al., 1994; Ødegaard, 2006; Pastorelli et al., 1997) and comparable to MBBR system operated under higher than design organic loading rates at 0.3 – 0.4 g-NH_4^+-N.m^{-2}.d^{-1} (Dulkadiroglu et al., 2005). This does suggest that nitrification activity is a function of organic loading rate, and it has been reported that the active biomass fraction is lower under high organic loads (Dulkadiroglu et al., 2005; Kampschreur, van der Star, et al., 2008). As such, the active biomass fraction in the sewer and CFBBR biofilm would be expected to be lower than biofilm and suspended growth systems grown under longer HRT's as with the MLSS systems. This would explain the higher specific nitrification rates of 7.02 g-NH_4^+-N.g-VSS^{-1}.d^{-1} observed for the 10.5 mg.L^{-1} MLSS culture.

Evidence of low active biomass fraction was also seen in the sewer biofilm, with the VS:TS fraction of 0.4 lower than 0.6 reported for rotating biological contactors and 0.78 for MBBR's (Andreottola et al., 2000; Martín-Cereceda et al., 2002). Despite this, the sewer biofilm seemed to outperform the CFBBR and 2850 mg.L^{-1} MLSS with average specific nitrification rates of 0.78 g-NH_4^+-N.g-VS^{-1}.d^{-1}. However, comparing to a 10.5 mg.L^{-1} MLSS culture with average specific nitrification rates of 7.02 g-NH_4^+-N.g-VSS^{-1}.d^{-1}, the sewer biofilm was outperformed. This was 100 times greater than observed with a 2850 mg.L^{-1} MLSS culture, likely due to increased substrate availability and DO concentration as a result of the lower biomass volume. The same is likely to be true for the sewer biofilm, where a very short HRT in the sewer perhaps resulted in periodic starvation of the biofilm, thus leaving some of the nitrifying population in dormant mode. When re-exposed to an ample supply of substrate for a prolonged time period in the specific nitrification rate tests under fully aerobic conditions, this could result in high specific nitrification rates.

Measurement of the VS content of the CFBBR biofilm was not possible due to the strong biofilm adhesion. As such, to allow comparison between all assays the mass of NH_4^+-N oxidised per unit volume of biomass assay was used to provide a standardised removal rate. With this applied the average specific nitrification rates are 183.7 g-NH_4^+-N.m^{-3}-assay.d^{-1} for 2850 mg.L^{-1} MLSS, 35.2 g-NH_4^+-N.m^{-3}-assay.d^{-1} for 10.5 mg.L^{-1} MLSS, 40.4 g-NH_4^+-N.m^{-3}-assay.d^{-1} for the CFBBR biofilm and 8.22 g-NH_4^+-N.m^{-3}-assay.d^{-1} for the sewer biofilm, agreeing well with the differing biomass quantities. It is therefore evident, that the CFBBR biofilm has comparable nitrification performance to its suspended growth counterpart, 10.5 mg.L^{-1} MLSS.

A 50 minute HRT allows increased time for accumulation of sludge and new microorganisms in comparison to shorter HRTs. As previously mentioned, biomass yield for heterotrophs is around 3.8 times higher than for autotrophs. As such, the concentration of substrate and oxygen diffused across the stagnant liquid film into the biofilm is higher than lower HRT's (Tchobanoglous et al., 2014a). With the high organic carbon load of settled sewage, this results in increased EPS production (Bassin et al., 2012), a thick heterotrophic layer, thick layer of inert solids encapsulated in the EPS and a thick stagnant liquid film layer (Figure 4.29). The autotrophic nitrifiers are subsequently out competed by the AH and less oxygen permeates to the nitrifying layer. This creates a deep layer of AH over the nitrifiers (Figure 4.29). By limiting HRT to 10 minutes, liquid velocity is higher and accumulation of sludge, new microorganisms and EPS is lower (Nogueira et al., 2002). The end result is a thinner biofilm and thinner stagnant liquid film (Figure 4.29) where the substrate flux rate is reportedly higher than for thick biofilms (Tchobanoglous et al., 2014a). As such, oxygen and substrate diffusion to the nitrifying layer occurs more freely, resulting in consistent nitrifying performance. Hence, a 10 minute HRT was determined to be a sustainable operating condition as after long term operation, average specific nitrification rates were comparable to the biofilm at a longer HRT of 50 minutes (Figure 4.10).

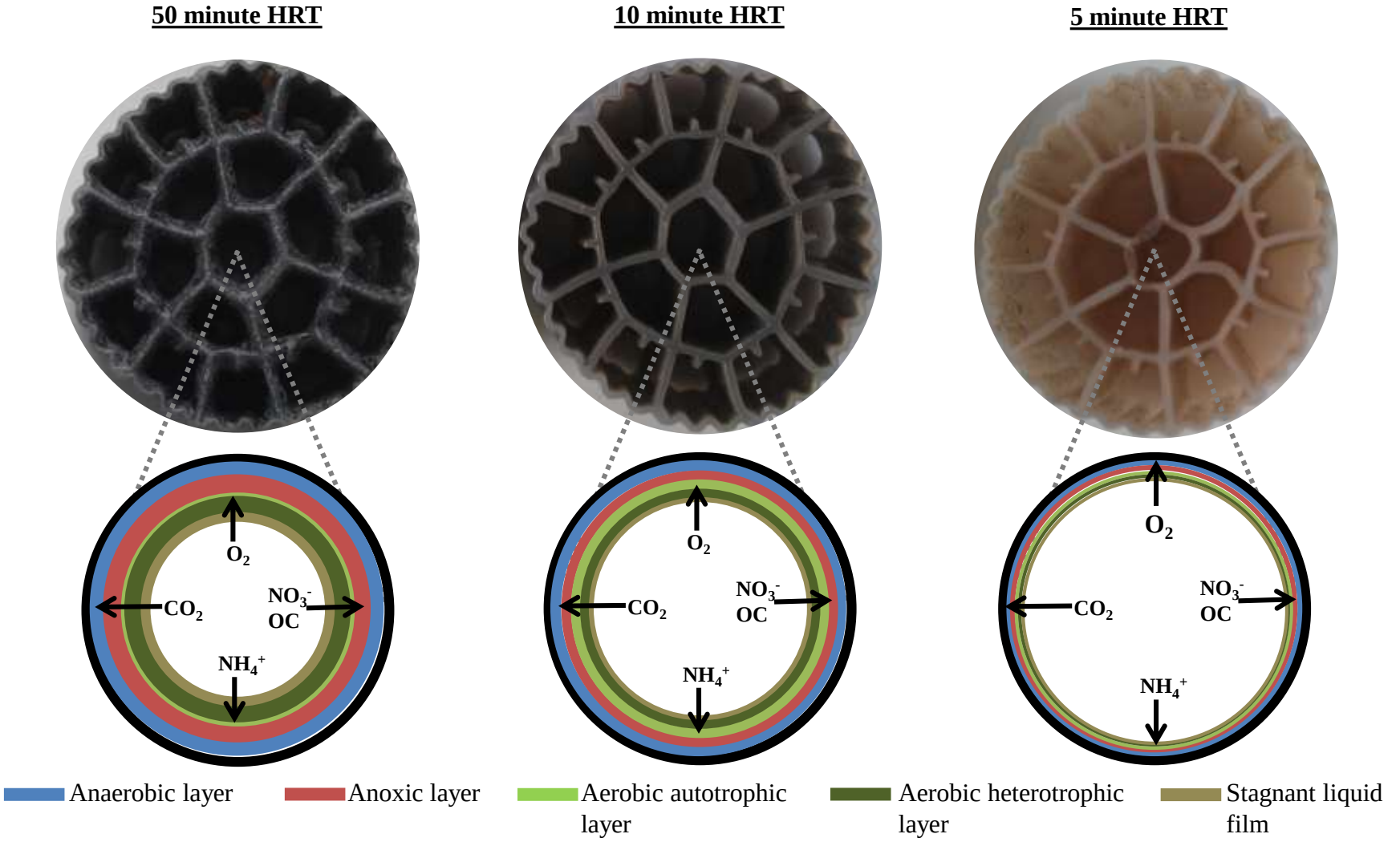


Figure 4.29 Biofilm attachment, substrate diffusion (OC is organic carbon) and oxygen diffusion as a function of HRT and loading rates to CFBBR carrier elements.

However, 10 minutes appeared to be very close to the actual limit, as reducing the HRT to 5 minutes resulted in a 32 % loss in nitrification performance after 50 to 60 days in the CFBBR biofilm. An HRT of 5 minutes potentially does not allow enough time for metabolism, resulting in a very thin under-developed biofilm (Figure 4.29). Deep oxygen permeation into the anaerobic and anoxic layers inhibits the anaerobes (Short et al., 2014), eventually resulting in detachment of the deep layers (Figure 4.29). At this point, the aerobic layers also begin to detach leading to a loss in nitrification performance.

Comparing this 5 minute HRT to the very low HRT of ~14 seconds in the 1 metre long sewer biofilm pipes would suggest that efficiency of nitrifying activity in the sewer biofilm would be expected to drop off over long term operation. This indeed agrees with other studies where it was demonstrated that nitrification reaction kinetics in the sewer biofilm were a strong function of wastewater composition, flow, level and velocity (Baban and Talinli, 2009; Ozer and Kasirga, 1995). These factors can lead to very high loading and shear stresses to the sewer biofilm (Nielsen et al., 1992; Vollertsen et al., 2005) limiting nitrification performance.

4.7.2 Nitrification inhibition assays in mixed cultures

It is well known that microbial communities will respond differently to toxic shocks, depending on their composition (McCarty, 1999; Ochoa-Herrera et al., 2011), biomass system type and length of exposure (Hu et al., 2004; Lee et al., 2009; Semerci and Ceçen, 2007; Sin et al., 2000). As such, tabulated values for nitrification inhibition are only indicative of a theoretical response of a mixed culture and are in fact highly dependent on the conditions under which they are conducted. For example, the reported copper (II) EC_{50} concentration for AS is in the broad range of 1.1 $mg.L^{-1}$ to 33 $mg.L^{-1}$ (Beyenal et al., 1997; Gutiérrez et al., 2002; Hayes et al., 1998; Ochoa-Herrera et al., 2011; Weon et al., 2004). As such, a rapid nitrification inhibition test and dose response analysis was conducted to evaluate the toxicity response of mixed cultures grown under the same substrate when conditioned as biofilms and suspended biomass (section 4.6.4a). A benchmark culture of 2850 $mg.L^{-1}$ MLSS was employed in this study, to allow comparison of all systems to a system typical of full-scale AS (Tchobanoglous et al., 2014d), for ATU, copper (II), chromium (VI) and nickel (II) shock loads. In all

cases, the 2850 mg.L^{-1} MLSS system demonstrated the greatest resilience to toxicity with respect to EC_{50} concentrations and percentage nitrification inhibitions (Table 4.7). To allow comparison of the CFBBR biofilm to a comparable suspended growth system, tests were conducted on an MLSS culture with an equivalent biomass concentration, namely the 10.5 mg.L^{-1} MLSS system.

The suspended growth systems were more resilient to ATU than the biofilm systems (Table 4.11). This was expected based on the greater sensitivity of the NH_4^+-N oxidation step by AOB to ATU shocks, than NO_2^- oxidation in step 3 (Butler et al., 2009; Figure 1.1). As the abundance of AOB was higher in MLSS systems than biofilm, shown by higher un-inhibited specific nitrification rates, this resulted in a lower ATU inhibitory effect on the bulk system.

Table 4.11 Expected and observed toxicity resilience between biofilm and comparable MLSS system (10.5 $mg.L^{-1}$ MLSS) in relation to nitrification inhibition, with explanations.

Toxicant	Inhibitory Mechanism	Most resilient system		Proposed Explanation	Evidence
		Expected	Observed		
ATU	- Inhibition to step 1 NH_4^+ oxidation (Butler et al., 2009) - AOB most susceptible (Butler et al., 2009)	MLSS	MLSS	AOB more abundant in MLSS than in biofilm, hence same concentration of ATU has lower effect on comparable MLSS assay than biofilm system.	- Higher un-inhibited specific nitrification rates in 10.5 $mg.L^{-1}$ MLSS than CFBBR biofilm - Dose response (Figure 4.13) and inhibitory effect comparison (Table 4.7)
Cu^{2+}	- Copper (II) binds to the membrane of cells through extracellular sorption (Lee et al., 2009) - NOB more susceptible than AOB (Stein and Klotz, 2011)	Biofilm	MLSS	AOB more abundant in MLSS than in biofilm; as a result, copper (II) was used as a catalyst for step 1 of nitrification (Figure 1.1) rather than shock NOB (Ochoa-Herrera et al., 2011).	- Dose response (Figure 4.12) and inhibitory effect comparison (Table 4.7)
Cr^{6+}	- Transported into cells, reduce to chromium (III) ions that then react with intracellular material (Martell, 1981) - - NOB more susceptible than AOB (Stein and Klotz, 2011)	Biofilm	Biofilm	Heterotrophs act as a protective layer to the nitrifiers (Bassin et al., 2012), allowing greater resilience of biofilm systems over suspended growth (Hayes et al., 1998; Lee et al., 2009; Weon et al., 2004).	- Dose response (Figure 4.15) and inhibitory effect comparison (Table 4.7)
Ni^{2+}	- Mode of action same as chromium (VI), but it remains at same valency (Cokgor et al., 2007) - NOB more susceptible than AOB (Stein and Klotz, 2011)	Biofilm	Biofilm	Same as chromium. Also, inhibitory effect is a strong function of substrate type (Cokgor et al., 2007), depending largely on the COD:Ni(II) ratio (Gikas, 2008), likely to be higher in 10.5 $mg.L^{-1}$ MLSS system than CFBBR biofilm.	- Dose response (Figure 4.14) and inhibitory effect comparison (Table 4.7)

Biofilm systems were expected to have a higher resilience to copper (II) shocks than suspended growth systems due to the protective AH layer (Bassin et al., 2012). This is broadly related to the diffusion limited conditions of a biofilm. The concentration of substrate, nutrients and indeed toxicants would be expected to be lower in the stagnant liquid film than bulk liquid, resulting in lower concentration fluxes into the biomass than suspended growth systems (Tchobanoglous et al., 2014a). Furthermore, it is believed that unlike other heavy metals copper (II) actually binds to the membrane, disrupting its structure (Avery et al., 1996; Hu et al., 2003, 2004; Lee et al., 2009; Sani et al., 2001), so the heterotrophic layer (Bassin et al., 2012) was expected to act as a barrier to toxicity. In reality, the suspended growth systems were much more resilient than biofilm systems (Table 4.11), with an improvement in nitrification performance on addition of copper (II) up to ~45 $mg.L^{-1}$. As copper (II) can act as a stimulant to the trans-membrane copper protein employed in step 1 (Stein and Klotz, 2011) the greater resilience in suspended growth systems was attributed to the higher AOB abundance than the biofilm systems.

As with copper (II), step 3 has been shown to be more sensitive to chromium (VI) toxicity than step 1 (Figure 1.1; Table 4.11; Stein and Klotz, 2011). However, unlike copper (II), the ions are transported across cell membranes, reduce to chromium (III) ions and disrupt cell structure through intracellular inactivation (Martell, 1981). Biofilm systems were again expected to be more resilient than suspended growth for the same reasons as with copper (II), as reported in the literature (Hayes et al., 1998; Lee et al., 2009; Weon et al., 2004) and indeed, this was observed in this study (Table 4.11). It was deduced that the protective heterotrophic layer (Bassin et al., 2012), likely to be present in the high organically loaded biofilm, slowed down diffusion of chromium (VI) from the stagnant liquid film to the nitrifying layer and was hence diffusion limited (Tchobanoglous et al., 2014a). Once chromium (VI) flux has reached the nitrifying layer, transport of metals across the cell membranes is slow (Hu et al., 2004). These are the most likely reasons why very high concentrations of chromium (VI) are required to inhibit microbial activity.

The inhibitory mechanism of nickel (II) is the same as chromium (VI) but its valency does not alter once inside the cell (Martell, 1981). Again, biofilm systems were expected to demonstrate higher resilience in comparison to equivalent suspended growth systems, and this was shown to be the case in this study, with the sewer biofilm demonstrating the greatest resilience. The reasons for this are the same as for chromium (VI), with the addition that the scale of inhibitory effect depends on the COD:Ni(II) ratio (Gikas, 2008). This was significantly higher in 10.5 $mg.L^{-1}$ MLSS than the biofilms, potentially contributing to the lower resilience of suspended growth systems (Figure 4.15; Table 4.11).

The findings here demonstrate how biomass concentration plays a major role in the culture's response to toxicity on the whole, demonstrated by the 2850 $mg.L^{-1}$ MLSS system having the greatest toxicity resilience. The biomass environment can also have a large weighting on the inhibitory effect of a particular toxicant. This needs to be considered when rationalising a toxicity response from the CFBBR biofilm, for an early warning to a suspended growth WwTW treatment process. Furthermore, COD in the sewer would be higher than at the secondary treatment process, which can affect metal speciation and inhibitory effect, again leading to a difference in response at the EWS device in the sewer.

4.7.3 Biofilm gaseous responses to toxic shock

The CFBBR biofilm's response to heavy metal toxicity was successfully detected through N_2O and CO_2 gaseous emissions. Although the response was not proportional to the nitrification inhibition expected in the off-line assays, there were clear changes in gas production patterns, with greater effects seen at the higher toxicant concentrations. Neither N_2O nor CO_2 production were impacted as a result of ATU shocks, in spite of the biofilm being inhibited based on nitrification assays.

Since ATU effectively inhibits ammonium oxidation (Butler et al., 2009) in step 1, a step involving both AOB and methanotrophs (Figure 1.1) as a result of their homologous copper membrane mono-oxygenase enzymes (McCarty, 1999; Stein and Klotz, 2011), DO would be expected to increase during inhibition to these aerobic organisms. However, if the relative abundance of these species is low in comparison to other aerobic species in the biofilm, their relative DO consumption would be low, and any changes to DO consumption of the biofilm as a whole would be marginal. Under 6.5 $mg.L^{-1}$ and 32 $mg.L^{-1}$ ATU shock loads a DO response was not recorded from the CFBBR biofilm and the CUSUM data tended to hover about zero. Conversely, for all CFBBR heavy metals shock tests, an increase in DO was observed during the shock duration indicating a decrease in microbial respiration rate. This potentially suggests another species other than AOB and methanotrophs is responsible for the bulk increase in DO observed during heavy metal toxicity. It is likely the species at work there was AH and as such, a DO response would not be expected with ATU as they are not sensitive to ATU (Butler et al., 2009).

Fitting with the nature of ATU inhibition to the first step of nitrification, the CFBBR biofilm's response could not be characterised through an N_2O emissions spike (Table 4.12). In terms of CO_2, no spike was evident which could be due to continuation of methanogenesis, not known to be affected by ATU (Figure 1.1; Capone et al., 1983; Sanchez et al., 1996). However, a small drop in CO_2 emissions was observed around 90 minutes into the ATU shock duration and the CUSUM data did indicate that these changes were significant. This could be linked to a drop in denitrification activity, as a direct result of less available NO_3^- (Figure 1.1; Table 4.12) following nitrification inhibition (Desloover et al., 2012).

Table 4.12 Expected and observed biofilm gaseous responses to toxicity, with explanations linked to figure 1.1.

Toxicant	Expected emissions pathway (Figure 1.1)	Toxicity gaseous response		Proposed explanation	Conclusion
		Expected	Observed		
ATU	- Step 1 inhibition (Butler et al., 2009) - No impact on HDN, AH or methanogens (Butler et al., 2009; Capone et al., 1983; Sanchez et al., 1996)	- Limited N_2O emissions - No change to CO_2 emissions	- No change in N_2O emissions (Figure 5.11) - Significant drop in CO_2 emissions 90 minutes into shock	- No impact on AH or HDN - Methanotrophs and AOB inhibited - Oxidation rate of NH_4^+ to NH_2OH in step 1 slows - Without NH_2OH intermediate step 2 and the pathways to N_2O production cannot ensue - CO_2 drop with lower denitrification activity due to less available NO_3^-	- Impact on N_2O generation was too low to be detected by monitor - ATU shocks are not reliably detected through gaseous responses
Copper (II)	- AH, methanogens and NOB inhibited (Barber and Stuckey, 2000; Ochoa-Herrera et al., 2011) - NH_2OH accumulation (Desloover et al., 2012; Stein and Klotz, 2011) - NO_2^- oxidation in Step 3 slows (Desloover et al., 2012) - NH_2OH reduction to NO by AOB and methanotrophs in step 2a (Desloover et al., 2012) - NOS inhibited by hypoxic environment due to reduced respiration rate of aerobes	- Increase in N_2O emission - Increase in CO_2 emission - Increase in DO	- Increase in N_2O and CO_2 emission (Figure 4.24a) - Emission intensity increased with increasing copper (II) concentration - CO_2 emission recovery period post shock - Sharp increase in DO	- Increase in N_2O and CO_2 emissions in response to toxicity as expected - Emissions pathways likely to be as expected - CO_2 fixation rate by AOB in step 1 (Berg, 2011), NOB (Oguz et al., 2006) in step 3 and use as an electron acceptor by methanogens in step 8 (Tchobanoglous et al., 2014b) increases when activity resumes post shock, remaining high until a steady state is reached - Reduction in heterotrophic activity once shock is removed as a delayed response	- Copper (II) shocks can be reliably detected through N_2O and CO_2 gaseous responses

	(Short et al., 2014) - Reduction in CO_2 fixation / consumption by NOB, AH and methanogens (Capone et al., 1983; Oguz et al., 2006; Sanchez et al., 1996; Tchobanoglous et al., 2014b)				
Chromium (VI)	- Same as copper (II) (Madoni et al., 1999)	- Increase in N_2O emission - Increase in CO_2 emission - Increase in DO	- Increase in N_2O and CO_2 emission (Figure 4.26a) - CO_2 emission recovery period post shock longer than with copper (II) - Lower DO increase than with copper (II)	- Heterotrophs and autotrophs have similar sensitivity to chromium (Madoni et al., 1999) .	- Chromium (VI) shocks can be reliably detected through N_2O and CO_2 gaseous responses - High concentrations are required due to slow transport across cell membranes - Slower biofilm recovery period than with copper (II)
Nickel (II)	- AOB inhibited (Hu et al., 2004) - Transient NH_4^+ accumulation (Chandran and Smets, 2000) - NOB inhibited by excess NH_4^+ (Chandran and Smets, 2000) - NOS inhibited by hypoxic environment due to reduced respiration rate of aerobes (Short et al., 2014)	- Increase in N_2O emission - Increase in CO_2 emission - Increase in DO	- Increase in N_2O and CO_2 emission (Figure 4.26a) - Emission intensity increase with higher Nickel (II) concentration - CO_2 emission recovery period post shock longer than with	- Cells likely take longer to recover than with copper (II), hence longer emissions recovery period - Biomass fraction of nitrifiers lower than heterotrophs (Bassin et al., 2012) - AH have lower sensitivity than nitrifiers to nickel (II), so DO response is low (Hu et al., 2004)	- Nickel (II) shocks can be reliably detected through N_2O and CO_2 gaseous responses - High concentrations are required due to slow transport across cell membranes

	copper (II)
- Reduction in CO_2 fixation / consumption by NOB, AH and methanogens (Capone et al., 1983; Oguz et al., 2006; Sanchez et al., 1996; Tchobanoglous et al., 2014b)	- Lower DO increase than with copper (II)

There was generally a positive correlation between toxicant concentration and gaseous emissions peak height / intensity as expected (Figure 1.1; Table 4.12). This was seen in the copper (II) shock tests, with the largest N_2O and CO_2 responses arising from a 96 $mg.L^{-1}$ copper (II) shock (Figure 4.24a). Likewise, gaseous emissions response of the CFBBR biofilm was evident at 290 $mg.L^{-1}$ chromium (VI), 33 $mg.L^{-1}$ nickel (II) and 131 $mg.L^{-1}$ nickel (II) shock loads. Responses to toxicity were also evident through DO spikes as expected (Table 4.12).

Conversely to the moderate differences in origins of N_2O, the evolution of CO_2 emissions followed broadly the same pathway for copper (II), chromium (VI) and nickel (II), and responses occurred as expected (Figure 1.1; Table 4.12). Unexpectedly, once the shock was removed, there appeared to be a recovery period post copper (II) shock, where CO_2 emissions remained low. The findings suggested the length of this microbial recovery period duration in relation to heavy metal toxicant was $Ni^{2+} > Cr^{6+} > Cu^{2+}$ and this agrees with reported studies (Hu et al., 2004). The reasons for this were again attributed to the inhibitory mechanism of chromium (VI) and nickel (II) set out in section 6.2 (Table 4.11). The apparent microbial recovery period, observed by a drop in CO_2 emissions, likely originated from a combination of:

- An increase in CO_2 fixation by AOB in step 1 (Berg, 2011) and NOB (Oguz et al., 2006) in step 3 when activity resumes post shock. Potentially, the bacteria operate at a higher rate, until a steady state is reached.
- An increase in CO_2 reduction by methanogens in step 8 (Capone et al., 1983; Sanchez et al., 1996) for the same reasons.
- A reduction in aerobic heterotrophic metabolism once the shock is removed, as a delayed response to toxicity. Heavy metals have been shown to inhibit AH responsible for removal of organic compounds (Gikas, 2008; Ochoa-Herrera et al., 2011; Vaiopoulou and Gikas, 2012). Likewise, HDN have been reported to be very sensitive to copper (II) (Ochoa-Herrera et al., 2011), chromium (VI) (Mazierski, 1994; Vaiopoulou and Gikas, 2012) and nickel (II) (Gikas, 2008).

The findings highlight the advantage of employing a mixed culture biofilm as part of an EWS, as the response of multiple species to a toxicant could be characterised with the same parameters (i.e. DO, N_2O and CO_2). The largest source of N_2O and CO_2 emissions

in response to toxicity was likely to be a multistep process leading to incomplete denitrification and a reduction in CO_2 fixation (Figure 1.1; Table 4.12). Realistically, a response can be detected for known toxicants of both autotrophs and heterotrophs such as copper (II), nickel (II) and chromium (VI), but toxicants specific to nitrification such as ATU are likely to be undetectable due to the inhibition pathway (Figure 1.1; Table 4.12).

CHAPTER 5. IMPLEMENTATION OF EARLY WARNING SYSTEM

The characterisation of biofilm development and the profiling of mixed culture responses to toxicity, including gas emissions, were used to develop an EWS for implementation by a water utility. Six options are proposed and appraised based on the desired risks, benefits and the associated whole life costs for each system. In this study, biofilm responses have been successfully characterised for heavy metals. As such, the biofilm based EWS would be most useful in sewer catchments accepting discharges from high risk industries such as mining, smelting, metallurgical, semiconductor manufacturing, electroplating, tanneries and metal finishing (Ochoa-Herrera et al., 2011; You et al., 2009) as well as potential landfill leachate carried to the sewer through surface run-off (Ceçen et al., 2010). The data from the toxic events could then be used to inform the management strategy at the water utility, treatment options as well as potential evidence to build a case against the offending customer (Love and Bott, 2000).

In addition to providing an early warning of a toxic event, it is important to obtain samples of the toxic sewage to verify its composition. This valuable evidence could help strengthen the prosecution case, as well as assist the decision on how to treat the toxic sewage at the WwTW.

5.1 Whole life costing

The whole life cost was analysed using Severn Trent's whole life costing tool with a reported methodology (Newton and Reid, 2007). The assumptions were; an asset life of 20 years, a CAPEX installation cost 25% of the total price, an August 2014 interest rate (consumer price index) of 0.5 % (Office for National Statistics, 2014), an inflation rate of 1.5 % (Bank of England, 2014), average electricity unit cost of 7.955 p.kWh^{-1} for manufacturing industry in quarter 1 of 2014 (GOV.UK, 2014) and a Severn Trent operator hourly rate of £26.50 (including salary, training and pension costs). Using these values along with equipment capital and consumable / overhaul costs (Table 5.1), six different EWS installations were analysed, namely:

A. **Basic biofilm based EWS;** One CFBBR based system at the inlet works and one in the sewer network. This represents the basic installation to give an in-network early warning and rationalise the response at the inlet works.

B. **Multiple location biofilm based EWS;** One CFBBR based system at the inlet works and two in the sewer network. This system represents an upgrade of system A, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works.

C. **Basic mixed EWS;** One Nitritox at the inlet works and one CFBBR based systems in the sewer network. This system represents an upgrade of system A, whereby an early warning can be provided in the network and rationalisation of that response with a percentage nitrification inhibition calculation by the Nitritox at the inlet.

D. **Multiple location mixed EWS;** One Nitritox at the inlet works and two CFBBR based systems in the sewer network. This system represents an upgrade of system C, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works.

E. **Basic CO_2 mixed EWS;** One Nitritox at the inlet works and one CFBBR based system (monitoring CO_2 only) in the sewer network. Findings from this study suggest a CFBBR based EWS can respond to the same toxicant spectrum employing only CO_2 monitoring and omitting the N-Tox N_2O monitor. This system was included to compare against system C.

F. **Multiple location CO_2 mixed EWS;** One Nitritox at the inlet works and two CFBBR based systems (monitoring CO_2 only) in the sewer network. This system represents an upgrade of system E, allowing an early warning from two points in the network and rationalisation of these responses at the inlet works. This system was included to compare against system D.

Table 5.1 Equipment capital (CAPEX) and operation expenditure (OPEX) for six EWS setups. Setup A; One CFBBR system at inlet and one in-sewer. Setup B; One CFBBR system at inlet and two in-sewer. Setup C; One Nitritox monitor at inlet and one CFBBR system in-sewer. Setup D; One Nitritox at inlet and two CFBBR systems in-sewer. Setup E; One Nitritox monitor at inlet and one CO_2 only CFBBR system in-sewer. Setup F; One Nitritox at inlet and two CO_2 only CFBBR systems in-sewer. The OPEX includes overhaul costs averaged over an asset lifetime of 20 years.

Equipment	Setup A		Setup B		Setup C		Setup D		Setup E		Setup F	
	CAPEX (£)	OPEX (£/year)	CAPEX (£)	OPEX (£/year)	CAPEX (£)	OPEX (£/year)	CAPEX (£)	OPEX (£/year)	CAPEX (£)	OPEX (£/year)	CAPEX (£)	OPEX (£/year)
N_2O monitor[a]	£36,000	£40	£54,000	£60	£18,000	£20	£36,000	£40	-	-	-	-
Nitritox[a]	-	-	-	-	£22,000	£1,040	£22,000	£1,040	£22,000	£1,040	£22,000	£1,040
CO_2 monitor[b]	£432	£43	£648	£65	£216	£22	£432	£43	£216	£22	£432	-
CO_2 sample pump[c]	£142	£28	£213	£43	£71	£14	£200	£28	£71	£14	£200	£28
CO_2 analysis cell[d]	£40	-	£60	-	£20	-	£40	-	£20	-	£40	-
Submersible pump[e]	£875	£188	£875	£188	£875	£188	£875	£188	£875	£188	£875	£188
Supply Tank[f]	£100	-	£150	-	£100	-	£150	-	£100	-	£150	-
Pipes[d]	£200	-	£300	-	£200	-	£300	-	£200	-	£300	-
Fixings[d]	£200	-	£300	-	£200	-	£300	-	£200	-	£300	-
Peristaltic pump[g]	£2,000	£213	£3,000	£320	£1,000	£107	£2,000	£213	£1,000	£107	£2,000	£213
CFBBR[d]	£413	£14	£620	£21	£207	£7	£414	£14	£207	£7	£414	£14
Aeration pump[h]	£150	£16	£225	£24	£75	£8	£150	£16	£75	£8	£150	£16
Pump for autosampler[i]	£400	£40	£600	£60	£400	£40	£600	£60	£400	£40	£600	£60
HMI / PLC[j]	£600	£120	£900	£180	£600	£120	£900	£180	£600	£120	£900	£180
Internet connection[k]	£60	£246	£90	£369	£60	£246	£90	£369	£60	£246	£90	£369
Pico data logger[l]	£318	£32	£477	£48	£318	£32	£477	£48	£318	£32	£477	£48
Labour*	£14,250	£879	£21,220	£1,291	£14,813	£2,010	£21,690	£2,422	£8,780	£2,007	£9,623	£2,417

[a](Dotro, 2009), [b](Vaisala, 2014), [c](RS online, 2014a), [d](Pipestock, 2014), [e](Pump Sales Direct, 2014), [f](Direct Water Tanks, 2014), [g](Fisher Scientific, 2014), [h](Machine Mart, 2014), [i](RS online, 2014b), [j](RS online, 2015), [k](H3G, 2014), [l](RS online, 2014c), *CAPEX labour costs were assumed to be 25% of the total cost.

5.1.1 Biofilm based EWS

The monitoring unit, including CFBBR, N-Tox, CO_2 monitor, feed pump, controlling computer, internet connection and an autosampler (Figure 5.1) should be deployed throughout a sewer network at key locations, perhaps as part of a real time control (RTC) study. The simplest form of EWS would require two units deployed: one in the sewer network on a high risk trader route and one at the WwTW inlet, to rationalise the response (Figure 5.1). Deployment to locations along a sewer pipeline is not recommended as the supply tube to the reactor has the potential to catch rags and promote build-up of fat deposits, posing a risk of sewer blockage (Appendix 2). The most suitable location for in-sewer deployment would be a sewage pumping station.

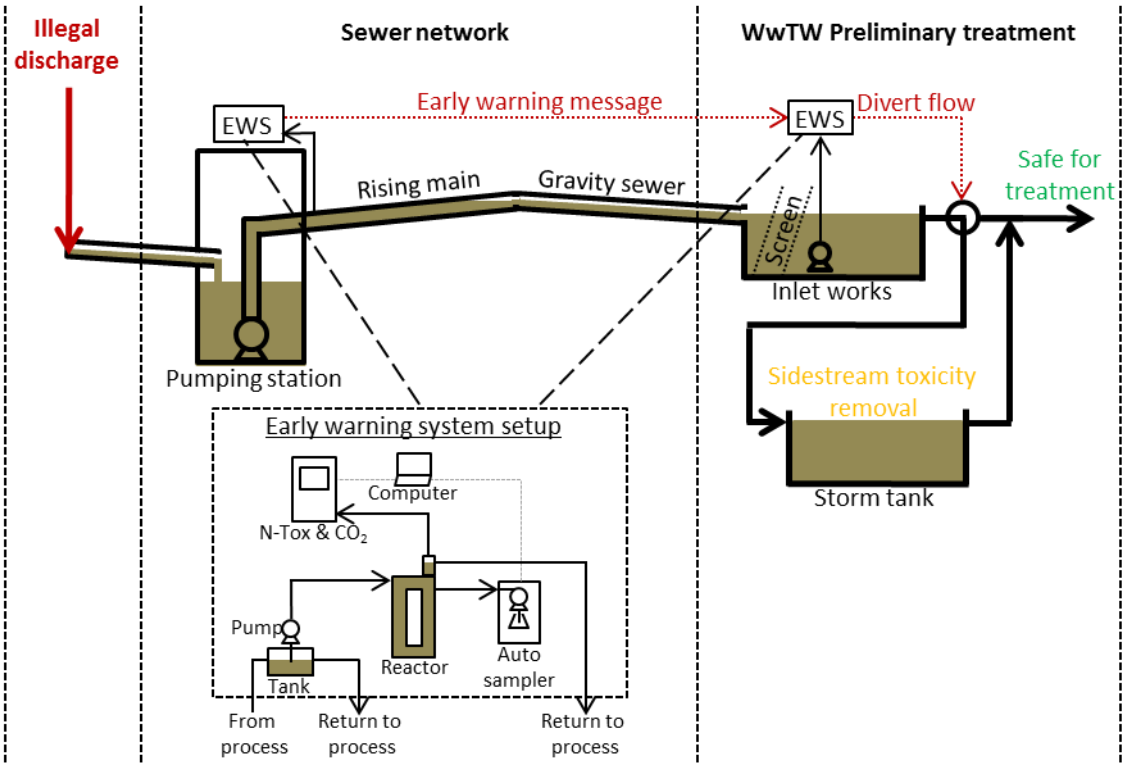


Figure 5.1 Early warning system implementation setup in a full-scale sewer and wastewater treatment process.

To deliver sewage to the CFBBR, a tapping point could be installed on the rising main, with a bore size of 25 mm. The pressurised sewage would be fed to a tank at the surface, with an overflow back to the pumping well. The overflow should have a bore size of 100 mm to reduce blockage risk and account for swell due to the pressurised feed. The reactor would then take its 0.7 $l.min^{-1}$ from this tank using a peristaltic pump and

overflow straight back into the pumping well. This method will remove the need for an additional lift pump for the EWS, reducing maintenance requirements. In addition, the tapping point bore size of 25 mm allows free passage of solids and small rags, whilst prohibiting larger objects from entering the EWS supply tank.

The device at the WwTW inlet can either be located right at the sewer mouth pre-screen or post screen, as long as its location is upstream of the flow diversion point to the storm tanks. If it is located pre-screen, the device will need a submersible cutter pump with strainer, to feed a tank at ground level, and the reactor will then take its feed from this tank using a peristaltic pump. If the device is located post screen, it may be possible to feed the reactor directly from the process using the peristaltic pump, with a strainer at the opening of the supply tube. In both instances, the device should be located at ground level, and overflow back into the process (Figure 5.1). The reactors receive a continuous feed of sewage with an HRT of 10 minutes, sustaining the biofilm and allowing a quick response. On detection of a biofilm response by the device in the sewer network, an early warning signal will be sent to the device at the inlet works and an in-sewer sample obtained from the supply tank by the auto-sampler system (Figure 5.1). An autosampler system can be achieved through digital outputs from the data logging equipment / software to control a small peristaltic pump during a toxic response.

As well as warning the inlet works device, the early warning signal should also be channelled to the operators at the WwTW via mobile phone alerts. Preparations should then be made to divert the incoming flow to the storm tank, with the lead time from the device in the sewer to WwTW inlet estimated based on the distance from the WwTW and flow into the WwTW at that time. At this point, the mitigation protocol will effectively be on standby as it is still possible the toxic sewage will be sufficiently diluted upon reaching the WwTW. However, if a response is detected by the device at the inlet after the estimated lead time for the toxic plug, the influent should be diverted to the storm tank, and the device will automatically collect a sample (Figure 5.1). The diversion can be terminated once the N_2O and CO_2 response peaks have dropped back to baseline.

The next steps at this point would be to analyse the samples to determine the composition and make steps to reduce the toxicity of the diverted influent. The options

for the management strategy have been reviewed in relation to WwTWs size and scale of toxicity in section 5.2.

The total capital cost for the CFBBR based EWS described above with two installations would be £56,986 (Table 5.2). Operational costs would be attributed to electricity to run the pumps and monitoring equipment, cleaning, replacing the peristaltic tube 4 times a year and general maintenance. The system does not require any consumables to sustain the culture, but instead uses the sewage as a substrate. The associated equipment and maintenance costs for different EWS installations are set out in section 5.1 (Table 5.1).

Table 5.2 Costs, risks and benefits of six EWS setups comparing capital and operation expenditure (CAPEX and OPEX respectively). Setup A; One CFBBR system at inlet and one in-sewer. Setup B; One CFBBR system at inlet and two in-sewer. Setup C; One Nitritox monitor at inlet and one CFBBR system in-sewer. Setup D; One Nitritox at inlet and two CFBBR systems in-sewer. Setup E; One Nitritox monitor at inlet and one CO_2 only CFBBR system in-sewer. Setup F; One Nitritox at inlet and two CO_2 only CFBBR systems in-sewer. An asset lifetime of 20 years was assumed.

Setup	Initial investment (CAPEX)	OPEX (£/year)			Whole life cost (£/year)	Benefits	Risks
		Operator time (hours/year)	Maintenance & overhaul	Energy			
A	£56,986	32	£1,764	£1,938	£6,511	- Lowest CAPEX and OPEX - Lowest whole life cost - No consumables required	- Single stage in-sewer toxicity validation risks false positive responses - Limited response time for mitigation of WwTW failure - Response limited to heavy metals
B	£83,677	47	£2,537	£2,325	£9,046	- Lower CAPEX and OPEX than setup D - Lower risk of false positives than A due to multi-stage toxicity validation - Longer lead time than A - No consumables required	- Highest energy costs - Response limited to heavy metals
C	£59,255	75	£4,069	£1,693	£8,725	- Able to respond to broader toxicant spectrum than CFBBR only system - Enumerates nitrification inhibition percentage, validating the toxic event	- Single stage in-sewer toxicity validation risks false positive responses - Limited response time for mitigation of WwTW failure - 30 minute sample frequency for Nitritox could miss toxic plug at WwTW - High chemical usage and operator time
D	£86,759	90	£4,841	£2,080	£11,259	- Lower energy requirements than 3 node CFBBR system - Broad toxicant spectrum - Enumerates nitrification inhibition percentage, validating the toxic event - Longer lead time than C	- 30 minute sample frequency for Nitritox could miss toxic plug at WwTW - High chemical usage and operator time - Highest whole life cost
E	£35,122	75	£4,050	£1,576	£7,383	- Same as C, responds to same spectrum - Lowest energy requirements	- Same as C
F	£38,492	90	£4804	£1,847	£8,575	- Same as D, responds to same spectrum	- Same as D

If more than one monitoring unit was installed within a real time controlled sewer network, i.e. expanding on the system described above, it may be possible to mitigate the risk of treatment failure. By reactively managing pumping stations and gates within the sewer network the lead time to the works along the effected sewer line could effectively be increased. This could allow more time for the toxicity of the sewage to be reduced through dilution into higher flows / volumes of non-toxic sewage. It may be possible with this setup to utilise in-network storage tanks to catch the toxic plug, and dilute the toxic sewage by trickle feeding back into the main stream. Such an EWS would demand an initial investment of £83,677 (Table 5.2).

It has been demonstrated that the biofilm based EWS is capable of detecting high toxicity level that would result in a high level of inhibition to the full scale treatment process (section 4.6.4). As such, setup A and B would be suitable to detect acute toxic events, but are not likely to respond to the lower toxicant concentrations that would cause chronic toxicity (Table 5.3). Setup A would only be suitable for small to medium sized WwTWs (≤10,000 & ≤100,000 PE) with small numbers of trade effluent routes and for catchments with at least 2 trade effluent routes setup B would be more suitable (Table 5.3). Setup B could also be expanded for very large catchments with multiple trade effluent routes, by increasing the number of in-sewer devices.

Table 5.3 EWS application decision matrix. Reviewed with respect to catchment size, watercourse type, scale of toxicity and toxic event type.

	Setup A	Setup B	Setup C	Setup D	Setup E	Setup F
Small and Medium WwTW ≤10,000 & ≤100,000 population	✓	✓	✓	✓	✓	✓
Large and Very Large WwTW ≤100,000 & ≤1,000,000 population	×	✓	×	✓	×	✓
Discharge to inland watercourse	✓	✓	✓	✓	✓	✓
Coastal discharge	✓	✓	✓	✓	✓	✓
High toxicant concentration / high inhibition at the WwTW inlet	✓	✓	✓	✓	✓	✓
Low toxicant concentration / high inhibition at the WwTW inlet	×	×	✓	✓	✓	✓
Acute toxic events	✓	✓	✓	✓	✓	✓
Chronic toxic events	×	×	✓	✓	✓	✓

5.1.2 Mixed EWS

The Nitritox was deemed impractical for use in the sewer network due to operation and maintenance requirements (section 4.6.1) but would be suitable for deployment at the inlet of a WwTW. Thus, an alternative to the CFBBR based system at the WwTW's inlet is a mixed EWS.

The total capital cost for an EWS employing a CFBBR system in the sewer network and a Nitritox at the inlet works would be similar to the CFBBR based EWS at £59,255 (Table 5.2), representing a 5 % increase in initial investment. Energy costs are 13 % lower but operator and maintenance costs are 134 % and 178 % higher respectively. The overall result is a whole life cost 37 % higher than a CFBBR only system (Table 5.2). The main advantages of this system are low energy requirements, a broader toxicant response spectrum than the CFBBR only EWS and calculation of a nitrification inhibition percentage to validate the toxic event (Table 5.2).

Finally the findings from this study suggest a CFBBR based EWS would be able to respond to the same toxicant spectrum employing only CO_2 monitoring (Figure 1.1; Table 4.12). As the CO_2 monitoring equipment was significantly less expensive than the N-Tox (Table 5.1) this would significantly reduce the CAPEX of a mixed EWS. As such, the total capital cost of a mixed EWS with one CFBBR based CO_2 only system in the sewer network and a Nitritox at the inlet works would be 40 % cheaper, at £35,122 and whole life cost would be 13 % cheaper (Table 5.2).

For detection of chronic toxic events the mixed EWS is more suitable than a CFBBR only system (Table 5.3). A Nitritox at the WwTWs inlet would be capable of detecting a wide toxicant spectrum over a broad concentration range, and warn of the lower concentrations that could lead to a chronic toxic event. The mixed EWS would be suitable for all sized catchments, but where two or more trade effluent routes exist, setup D or E would be required (Table 5.3). Again, the systems can be expanded for catchments with multiple trade effluent routes through addition of more in-sewer devices.

5.2 Management of a toxic event

In the UK, high risk traders are permitted to discharge effluent containing the heavy metals tested in this study at low concentrations (Table 5.4). To present a risk to the WwTW, traders would need to discharge very high concentrations of toxicant. The levels of copper (II), chromium (VI) and nickel (II) required at the inlet works to result in treatment inhibition has been calculated at DWF, flow to full treatment (FFT) and formula A flow for different sized WwTW (method described below). The subsequent management strategy has been proposed in relation to WwTW size.

Table 5.4 Examples of known toxicant trade effluent discharge consents for high risk traders in the UK.

			Trade effluent consent			
Trader	**Industry**	**Sewerage undertaker**	**Volume $m^3.d^{-1}$**	**Cr^{6+} $mg.L^{-1}$**	**Ni^{2+} $mg.L^{-1}$**	**Cu^{2+} $mg.L^{-1}$**
Red Industries Ltd	Waste management	Severn Trent Water Ltd	300	2.5	-	5.0
Solway Foods Ltd	Food processing	Severn Trent Water Ltd	750	-	-	-
Rapier Energy Ltd	Waste management / recycling	Northumbrian Water Ltd	200	3.0	-	3.0
Frogson Waste Management Ltd	Waste oil disposal	Yorkshire Water Services Ltd	100	1.0	3.0	2.0
Avanti Environmental Ltd	Waste management / recycling	United Utilities Plc	100	2.5	2.5	3.0

The concentration of heavy metals required to inhibit the treatment process and the required volume of water required to dilute toxic wastewater to a concentration causing no treatment inhibition has been assessed at three different influent flow conditions as follows:

Condition 1: DWF

- Flow into the WwTW under dry weather conditions
- Summation of domestic population (P), per capita flow (G, 150 $l.head^{-1}.d^{-1}$), infiltration (I, 30% of PG) and trade effluent flow (E, assumed 10% of PG)
- Calculated as DWF = PG + I + E (United Utilities Plc, 2011)

Condition 2: FFT

- The design maximum flow passed to full treatment, and maximum consented discharge flow of the WwTW
- Allows for rainfall

- Calculated as FFT = 3PG + I + 3E (United Utilities Plc, 2011)

Condition 3: Formula A

- Storm flow
- Difference between Formula A and FFT is passed to the storm tanks
- Calculated as Formula A = 1.36P + DWF + 2E (United Utilities Plc, 2011)
- The storm tanks were assumed to be designed for a per capita storm flow of 68 $l.head^{-1}.d^{-1}$ (Severn Trent Water, 2009b). At formula A flow into the WwTW, storm tanks sized this way provide 1.5 hours retention time before spilling to the watercourse.

5.2.1 Copper (II) shock

It has been demonstrated that nitrification in the 2850 $mg.L^{-1}$ MLSS system (representing a real ASP) is inhibited by a copper (II) concentration over 40 $mg.L^{-1}$ (Figure 4.12). To cause a 50% nitrification inhibition to the 2850 $mg.L^{-1}$ MLSS system, copper (II) concentration must be in excess of 86.9 $mg.L^{-1}$ (Figure 4.12) in the crude sewage at the inlet (before any storm separation). This concentration fully inhibits the CFBBR biofilm (Figure 4.12), and hence would be detectable through gaseous emissions.

Using a 250,000 PE works as an example, a copper (II) mass flow rate of 190 $kg.h^{-1}$ is required to achieve a copper (II) concentration of 86.9 $mg.L^{-1}$ at the inlet under DWF conditions (Figure 5.2). To achieve the same level of inhibition at FFT conditions, the required copper (II) mass flow rate almost triples to 489 $kg.h^{-1}$.

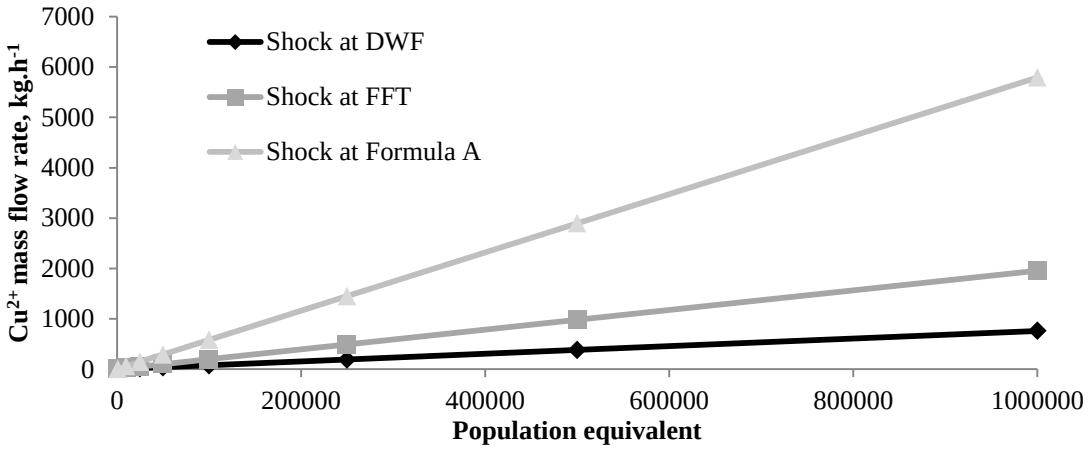


Figure 5.2 Mass flow rate of copper (II) in crude sewage at the inlet (before storm separation) required to result in a 50% nitrification inhibition of the 2850 mg.L^{-1} MLSS system.

Under storm conditions, i.e. formula A flow, the mass flow rate before storm separation required to achieve an 86.9 mg.L^{-1} shock is 8 times higher than at DWF. In these conditions, the flow passed forward to treatment is FFT (5625 $m^3.h^{-1}$) with the difference between formula A and FFT (11042 $m^3.h^{-1}$) passed to the storm tanks. Hence, the mass flow rate passed forward to treatment is the same as a shock under FFT conditions. The required flow rate of dilution water, to reduce the copper (II) concentration to 40 mg.L^{-1} in the flow passed forward to treatment is 6595 $m^3.h^{-1}$ (Figure 5.3). By comparison, a shock under DWF conditions requires 2565 $m^3.h^{-1}$ dilution water (Figure 5.3).

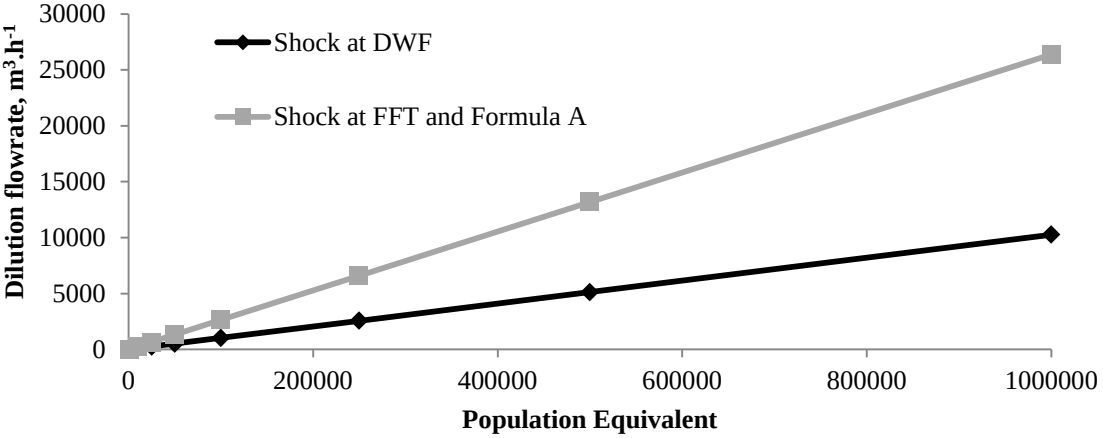


Figure 5.3 Dilution water flowrate required to reduce the copper (II) concentration to 40 mg.L^{-1} in the flow passed forward to treatment.

5.2.2 Chromium (VI) shock

The 2850 mg.L^{-1} MLSS system is inhibited by a chromium (VI) concentration over 32 mg.L^{-1} (Figure 4.15). To exhibit a 50% nitrification inhibition to the 2850 mg.L^{-1} MLSS system, a chromium (VI) concentration of 404.0 mg.L^{-1} is required (Figure 4.15). To achieve this at a 250,000 PE WwTW, a mass flow rate of 884 kg.h^{-1}, 2273 kg.h^{-1} and 6733 kg.h^{-1} under DWF, FFT and formula A conditions respectively is required (Figure 5.4). The required dilution water flowrate for a shock at DWF is 25430 m^3.h^{-1} and 65391 m^3.h^{-1} for a shock at FFT and formula A (Figure 5.5).

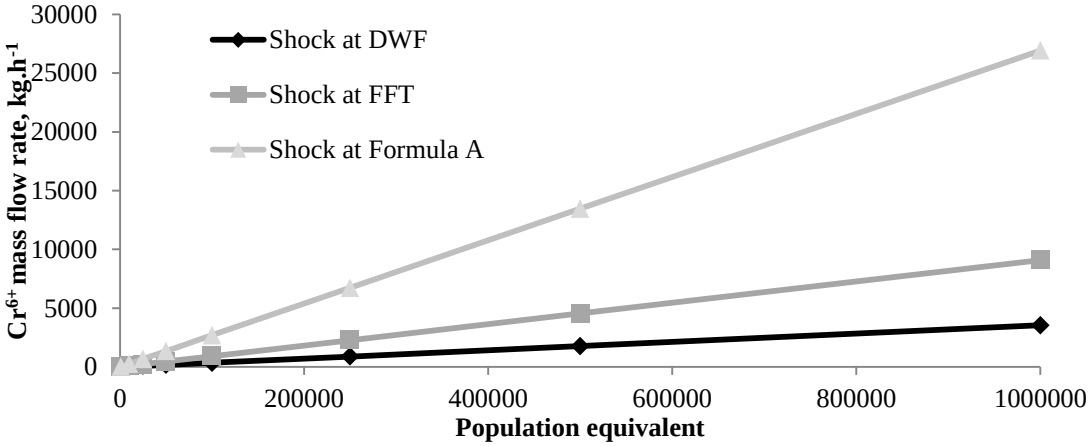


Figure 5.4 Mass flow rate of chromium (VI) in crude sewage at the inlet (before storm separation) required to result in a 50% nitrification inhibition of the 2850 mg.L^{-1} MLSS system.

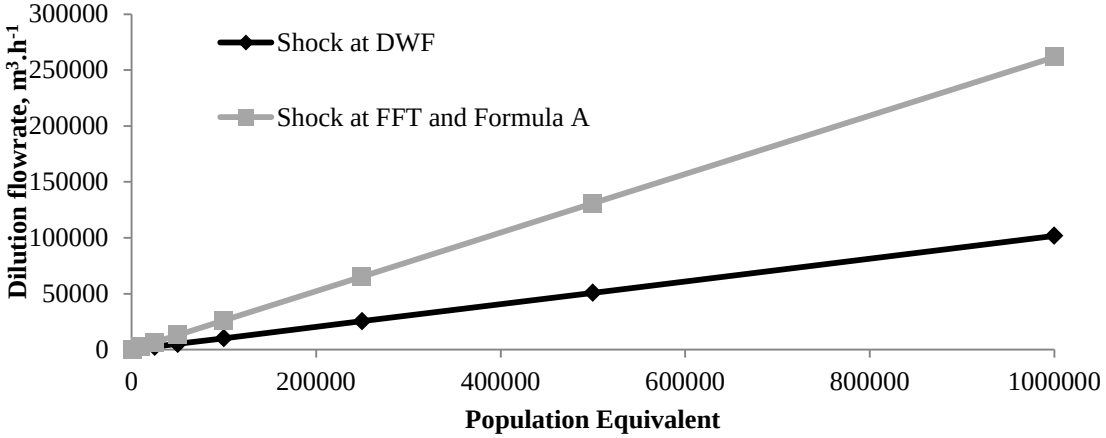


Figure 5.5 Dilution water flowrate required to reduce the chromium (VI) concentration to 40 mg.L^{-1} in the flow passed forward to treatment.

5.2.3 Nickel (II) shock

The 2850 mg.L^{-1} MLSS system is inhibited by a nickel (II) concentration over 16 mg.L^{-1} (Figure 4.14). To exhibit a 50% nitrification inhibition to the 2850 mg.L^{-1} MLSS system, a nickel (II) concentration of 211.4 mg.L^{-1} is required (Figure 4.14). To achieve this at a 250,000 PE WwTW, a mass flow rate of 462 kg.h^{-1}, 1187 kg.h^{-1} and 3517 kg.h^{-1} under DWF, FFT and formula A conditions respectively is required (Figure 5.6). The required dilution water flowrate for a shock at DWF is 26660 m^3.h^{-1} and 68555 m^3.h^{-1} for a shock at FFT and formula A (Figure 5.7).

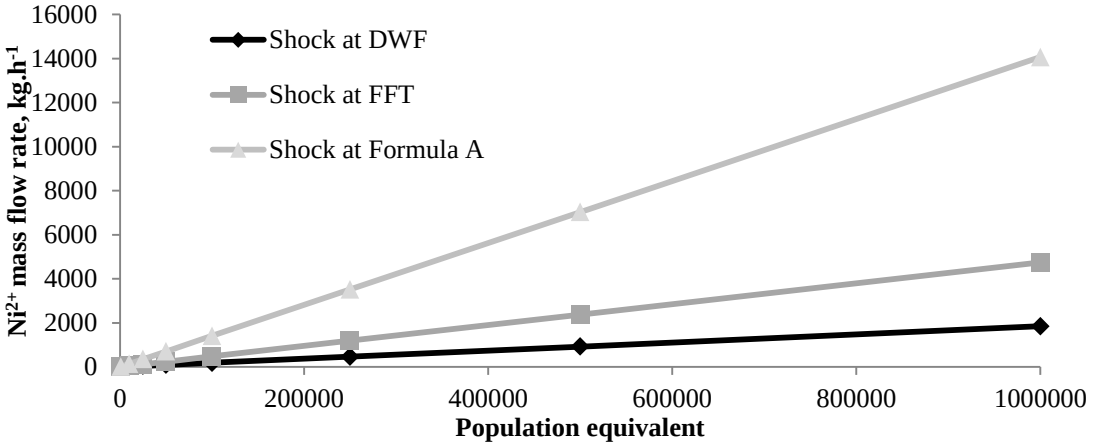


Figure 5.6 Mass flow rate of nickel (II) in crude sewage at the inlet (before storm separation) required to result in a 50% nitrification inhibition of the 2850 mg.L^{-1} MLSS system.

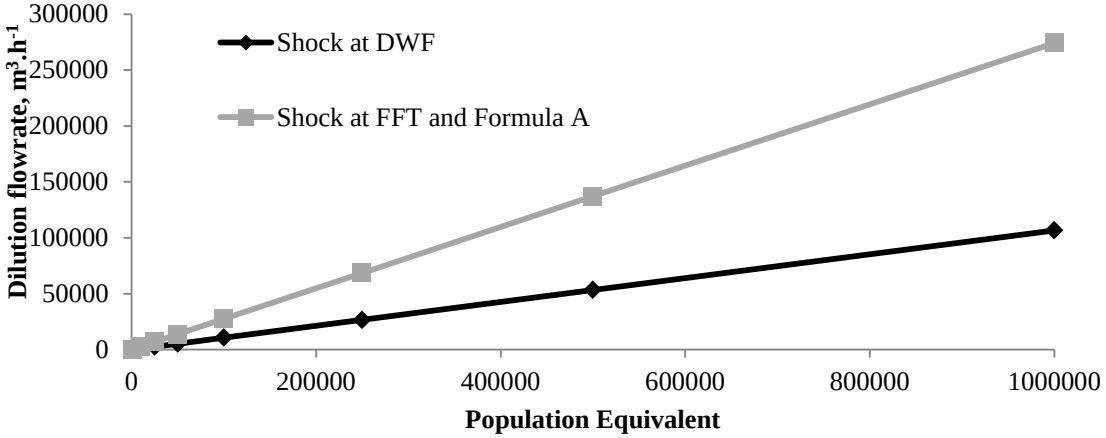


Figure 5.7 Dilution water flowrate required to reduce the nickel (II) concentration to 40 mg.L^{-1} in the flow passed forward to treatment.

5.2.4 Selecting a management strategy

A number of options are available for the management strategy, and these have been reviewed in relation to WwTWs size and toxicity level. They include:

1) Sufficiently dilute the toxic sewage in the storm tank either with potable water or final effluent. This can then be fed into the treatment process.
2) Trickle feed the polluted wastewater back into the treatment process.
3) Where appropriate based on characteristics, volumes and proximity to another works, transport contaminated wastewater to a larger works for treatment.
4) Where treatment is unfeasible, dispose of contaminated sewage to a licensed hazardous waste facility.

Sections 5.2.1 to 5.2.3 demonstrate that installing a system to dilute toxicity would potentially only be viable for a DWF toxic event at small (≤10,000 population) and medium sized (≤100,000 population) WwTWs (Table 5.5). At formula A flow the storm tanks have a retention time of 1.5 hours before spilling, and hence dilution would cause the storm tanks to spill prematurely. Therefore, due to the careful management required, mitigation of a toxic event through dilution with potable water or final effluent is not recommended (Table 5.5).

Table 5.5 Toxic event management strategy decision matrix.

	Small and Medium WwTW ≤10,000 & ≤100,000 population	**Large and Very Large WwTW ≥100,000 & ≥1,000,000 population**
DWF toxic event likely?	✓	✓
FFT toxic event likely?	✓	✓
Formula A toxic event likely?	✓	×
Dilution of DWF toxic event	✓ However, not recommended	×
Dilution of FFT toxic event	×	×
Dilution of Formula A toxic event	×	×
Tanker removal of captured toxic sewage	✓ FFT and Formula A shocks	×
At influent flow of ≤0.8FFT, dilute toxic sewage by storm tank recirculation	✓ 0.08DWF at DWF 0.16DWF at 0.8FFT	✓ 0.08DWF at DWF 0.16DWF at 0.8FFT

At small and medium sized WwTWs there is a risk that a trader could be capable of shocking the treatment process at FFT and formula A flows (Table 5.5). However, dilution flowrate requirement is not practical and at formula A it would not be permitted

as it would result in a failure of the FFT consent. The most workable solution in the case of a toxic shock at formula A flow would be to retain the spiked sewage within the storm tank, and tanker away to a larger WwTW (Table 5.5).

At large (≥100,000 population) and very large (≥1,000,000 population) WwTWs, a trader is not likely to be capable of discharging a mass flow rate sufficient to cause a 50% inhibition at formula A flows (Table 5.5). In the event this did happen, mitigation of inhibition would be very difficult, and would require dedicated storage should formula A shocks be the design condition. A more sensible approach would be to disregard inhibition at formula A for this sized WwTW and design a mitigation system for inhibition at FFT (Table 5.5).

5.2.5 Management strategy control philosophy

On detection / rationalisation of a toxic shock at the inlet works, the EWS should send a signal to the modulating penstock / flow meter upstream of the inlet screens, to close the modulating penstock and pass all flow to the storm tank. The retention time of the storm tank at the influent flow at that time should be calculated, with no more than that passed to the storm tanks.

Once the inlet works monitor has determined the end of the shock (drop in emissions), the toxic water can be returned to the inlet works from the storm tank at a maximum flowrate of 0.5DWF (Severn Trent Water, 2009b). The maximum crude sewage flow storm water can be returned into is 0.8FFT (Severn Trent Water, 2009b).

At DWF conditions, to ensure no additional dilution water is required, the modulating penstock should be controlled, such that no more than 0.08DWF of the storm tank contents are passed to treatment (Table 5.5). This will promote recirculation over the storm separation weir back into the storm tank, and will take around 97 hours to pass all contents of the storm tanks (if they are full) to treatment. At 0.8FFT conditions, the modulating penstock should be controlled such that no more than 0.16DWF of the storm tank contents are passed to treatment (Table 5.5). At this return rate, it will take 49 hours to pass all contents of the storm tanks (if they are full) to treatment.

Level in the storm tanks should be monitored, and the modulating penstock controlled such that it fully opens to allow FFT to pass to treatment if the level begins to rise due

to rainfall. This will prevent premature spillage of the storm tank contents. If severe rain occurs during the recirculation period there is a risk that there will not be enough capacity to retain the rainfall and the toxic sewage. As such, spillage of the toxic sewage is a risk under these conditions and it would be very difficult to mitigate pollution of the receiving water course. Thus, the option of diluting the toxic sewage with potable water or treated final effluent was explored in sections 5.2.1, 5.2.2 and 5.2.3.

5.3 Rationale for EWS implementation

5.3.1 Implications of toxic events

At the point of a toxic event, negative attention is immediately drawn to the water company despite them not actually being responsible for the toxic wastewater (The Guardian, 2009; The Independent, 2009; The Telegraph, 2009). Thus, along with an expensive clean-up operation and re-seed of treatment processes, many man hours need to be spent on damage control of public perception following a toxic event.

Where a toxic event results in a pollution incident such as a fish-kill and the offender is prosecuted, fines can run into the millions. In 2001, a trader in the USA was fined $14 million as a result of the 1999 White River cyanide fish-kill (US Department of Justice, 2001). However, it is difficult to reach such a verdict without compelling evidence, and in some cases the prosecution fails, as with the 2009 Strongford cyanide incident (BBC News, 2014; Burton Mail, 2014) and a smaller scale incident in Yorkshire (HFL Risk, 2011; Huddersfield Examiner, 2011). In the UK, the Environment Agency have urged the courts to issue larger fines as a stronger deterrent against events like this and stated they will continue to actively prosecute anyone who pollutes the environment (Environment Agency, 2009). Hence, identification of toxic events could help the prosecution case and deter from future illegal discharges as a result.

5.3.2 Sustainability of in-sewer EWS for acute toxicity detection

To assess the payback period of an in-sewer EWS, the Strongford pollution incident in 2009 has been used as an example of an acute toxic event. Strongford is a large inland works, with a PE of 342,000. The clean-up operation took 5 days and involved 200 tanker movements to transport 2 million litres of sewage to Minworth, Severn Trent Water's largest WwTW (Dotro, 2009). The range of a tanker on a full tank of diesel

(400 litres) is taken to be 435 miles (Scania AM, 2009). The price of a gallon of diesel was based on the October 2009 average price of a UK gallon of diesel in the West Midlands at 481.43 pence per gallon (AA, 2009). The fuel consumption was thus estimated as:

- Fuel consumption = vehicle range ÷ volume of fuel tank
  = 434.96 ÷ 400 = 1.09 miles/litre
- Miles per $gallon_{UK}$ (MPG_{UK}) = 1.09 x 4.55 = 4.94 MPG_{UK}
- Total fuel consumed = Total distance covered ÷ MPG_{UK}
  = (44.9 x 200) ÷ 4.94 = 1816.17 $Gallons_{UK}$
- Total fuel cost = Total fuel consumed x price of one gallon
  = 1816.17 x 481.43p = £8743.59

The total labour cost for the 50 Severn Trent Employees involved in the clean-up operation (Dotro, 2009) was £49,688, based on five 7.5 hour working days at the Severn Trent hourly rate of £26.50 set out in section 5.1. Hence the total clean-up cost of the Strongford pollution incident was £58,432. Based on this, pay back on installation of system A, C and E would require 2, 2.7, and 2.4 incidents to occur in the 20 year EWS lifetime respectively.

From this event, the clean-up cost per capita has been estimated at £0.14 in relation to labour costs and £0.17 overall. Applying this, the relative clean-up cost and payback of an in-sewer EWS has been estimated for various sized works (Table 5.6) with respect to the number of Strongford scale toxic events that would need to occur in order to pay back the 20 year whole life cost (Table 5.2) of the EWS.

Table 5.6 Estimated toxic event clean-up costs based on sewer catchment population equivalent and subsequent payback requirements for all six EWS systems.

Population equivalent	Clean-up cost	Number of incidents required for payback for all EWS systems					
		A	B	C	D	E	F
10000	£1,700	64.5	94.6	94.2	120.2	82.0	95.6
25000	£4,250	25.8	37.9	37.7	48.1	32.8	38.3
50000	£8,500	12.9	18.9	18.8	24.0	16.4	19.1
100000	£17,000	6.4	9.5	9.4	12.0	8.2	9.6
250000	£42,500	2.6	3.8	3.8	4.8	3.3	3.8
500000	£85,000	1.3	1.9	1.9	2.4	1.6	1.9
1000000	£170,000	0.6	0.9	0.9	1.2	0.8	1.0

From this, a trade-off was constructed and it was deduced that installation of an in-sewer EWS would only be viable in catchments larger than 200,000 PE (Figure 5.8).

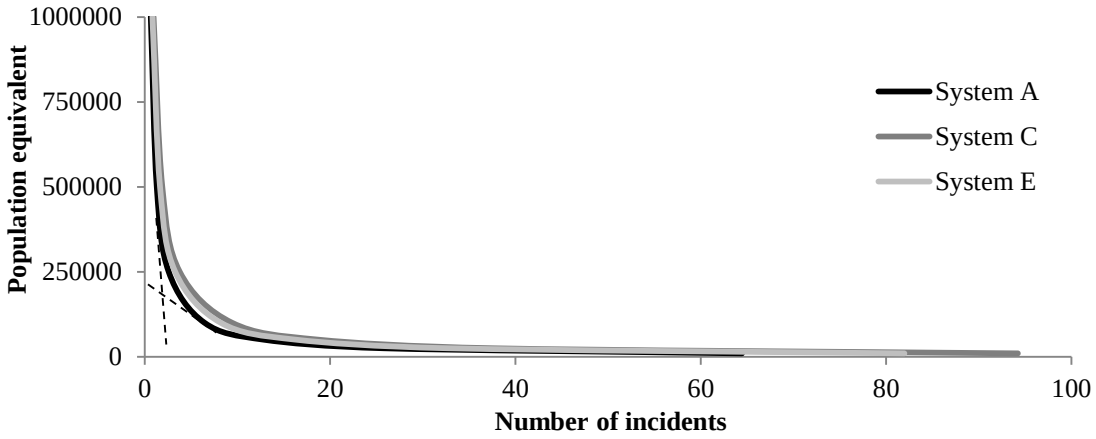


Figure 5.8 Trade-off for installation of an in-sewer EWS in various sized catchments with respect to the number of toxic incidents required to pay back the 20 year whole life cost of system A, C and E.

5.3.3 Clean-up costs for a range of incident scales

The Strongford incident occurred during a low flow period so the clean-up costs are not representative of toxic events during high flow periods. Based on section 5.3.2, the relative clean-up costs for DWF, FFT and formula A acute toxic events with a 1 hour duration has been estimated for a range of PEs (Table 5.7).

Table 5.7 Clean-up cost of an acute toxic event of 1 hour duration for a range of population equivalents.

Population equivalent	DWF	FFT	Formula A
10,000	£332	£332	£332
25,000	£2,025	£2,241	£3,106
50,000	£5,206	£5,638	£7,583
100,000	£10,219	£11,083	£15,190
250,000	£20,461	£21,974	£30,188
500,000	£50,756	£54,863	£75,615
1,000,000	£101,536	£109,534	£151,037

This was based on a nominal labour cost of £0.14 per capita and the number of tanker movements. Fuel costs were based on the number of 30 m^3 tanker movements over 44.9 miles (same as the Strongford incident). As coastal treatment processes are typically not designed to nitrify (i.e. no stipulated ammonia discharge consent), the resilience to toxicity is likely to be higher (section 4.7.2), and hence the required toxicant concentration for treatment inhibition is higher. However, this only changes the toxicant strength not the volume of toxic wastewater, therefore the clean-up cost of comparable toxic events will be the same for inland and coastal WwTWs.

5.3.4 Sustainability of in-sewer EWS for chronic toxicity detection

Long term exposure to sub-lethal toxicant concentration could lead to a gradual drop in treatment performance and eventual treatment failure. Chronic toxic events like this are likely to result in lasting inhibition of treatment performance and environmental damage, potentially leading to a drop in biodiversity, plant life and ecological population numbers (Section 1.8). Chronic events are currently detected through final effluent quality monitoring, paying close attention to treatment performance trends. However, to guarantee zero failures and mitigate chronic toxic events caused by sub-lethal toxicity, an EWS is essential. As demonstrated in section 5.1.2, a biofilm only EWS would potentially miss sub-lethal concentrations but a mixed EWS incorporating a Nitritox at the inlet works can respond to low levels of toxicity.

As previously mentioned, an EWS would be viable in catchments >200,000 PE based on the number of events required to pay back whole life costs. For smaller catchments with little or no industrial discharges, the risk toxicity leading to treatment failure is low. However, these catchments can be subjected to illegal fly tipping into sewer manholes and accidental spillages, which can have the potential to result in treatment inhibition at the WwTW. The high ODI's related to compliance with discharge consents (section 1.10) demonstrates the commitment many of the UK's wastewater treatment companies have on zero treatment failures. Hence, in light of the high ODIs for environmental compliance an EWS could be viable even when there is a low risk of toxicity.

CHAPTER 6. CONCLUSIONS

A nitrifying culture can be established in the sewer pipe wall biofilm with a growth time of 13 days. When growing biofilm in an organic-rich feed at a minimum of 10 minutes HRT, the nitrifying community in a CFBBR reaches a steady state at 180 days, with average specific nitrification rates of ~0.40 g-NH_4^+-N.m^{-2}.d^{-1}, comparable to pilot scale MBBR systems. By limiting HRT to 10 minutes, higher liquid velocities were possible along with lower accumulation of sludge, new microbes and EPS. This resulted in a thin biofilm and thin stagnant liquid film, aiding oxygen and substrate diffusion to the nitrifying layer for consistent nitrifying performance.

Each assay responded differently to toxicity. The inhibitory effect of the tested toxicants was Cu^{2+} > ATU > Ni^{2+} > Cr^{6+} for the CFBBR reactor biofilm. A similar trend was observed in MLSS and sewer biofilm systems whereby ATU > Cu^{2+} > Ni^{2+} > Cr^{6+} for 2850 mg.L^{-1} MLSS, ATU > Ni^{2+} > Cr^{6+} > Cu^{2+} for the 10.5 mg L^{-1} MLSS and ATU > Cu^{2+} > Cr^{6+} > Ni^{2+} for the sewer biofilm.

The biofilms had a comparable sensitivity to ATU shocks, were more sensitive to shocks with copper (II) but less sensitive to chromium (VI) and nickel (II) than the equivalent suspended growth biomass. The likely reasons for this were:

- Copper (II) concentrations up to 45 mg.L^{-1} acting as a stimulant to nitrification activity of the suspended growth systems, resulting in an overall lower inhibitory effect to the bulk culture than with biofilms.
- Slow transport of chromium (VI) and nickel (II) across cell membranes coupled with diffusion limitation from the stagnant liquid film across the protective heterotrophic layer to the nitrifying layer, meaning very high concentrations of these metals were required to overcome this.

The response of a biofilm to heavy metals can be characterised through N_2O and CO_2 emissions, utilising a CFBBR as part of an EWS. There was generally a positive correlation between toxicant concentration and gaseous emissions peak height / intensity, and a post shock emissions recovery period was observed agreeing with the trend Ni^{2+} > Cr^{6+} > Cu^{2+} in reported studies. Again, slow transport of chromium (VI)

and nickel (II) across cell membranes was the likely reason why a longer recovery period was observed via CO_2 emissions in comparison to copper (II) shock tests.

A basic installation utilising the Nitritox as an inlet works monitor (i.e. mixed EWS) would demand operator and maintenance costs 134 % and 178 % higher than a CFBBR only system respectively. This equates to a whole life cost 37 % higher than a CFBBR only system. However, the significant advantages of this setup are:

- Rapid toxicity early warning in the sewer network using the CFBBR system.
- The ability to validate that response, by measuring the actual nitrification inhibition percentage of the wastewater at the inlet to the works.
- Ability to detect acute and chronic toxicity
- In this sense, it is potentially more robust than a CFBBR only EWS.

Building on this, there is potential to just monitor CO_2 from the in-sewer CFBBR, but maintain the same toxicant spectrum as a system monitoring N_2O as well. Employing this setup would reduce the whole life cost of a mixed EWS by 13 %, bringing the costs closer to a CFBBR only EWS.

The low HRT in the CFBBR systems employed in this study may have led to lower sensitivity of the biofilm to heavy metal toxicity, particularly for chromium (VI) and nickel (II). To achieve a 10 minute HRT, the CFBBR volume was limited to 7 litres, due to the available pumps for bench scale testing. If lower sensitivity than MLSS systems is anticipated to be an issue in a full-scale EWS, a larger feed pump could be selected, to permit a larger CFBBR volume. The larger culture size would emit a higher mass of N_2O and CO_2 into the headspace. If the headspace was kept at the same size as the small test CFBBRs in this study the concentration of N_2O and CO_2 would increase. This would effectively amplify the signal, increasing the system's sensitivity to lower levels of toxicity.

Finally, an in-sewer EWS was shown to be viable in catchments larger than 200,000 PE owing to a trade-off between EWS whole life cost and the number of toxic events required in its lifetime for sustainable investment. However, in light of the high ODIs for environmental compliance an EWS could be viable in smaller catchments even when there is a low risk of toxicity.

CHAPTER 7. FUTURE RESEARCH

The monitoring technique employed in this study has offered a method of on-line wastewater toxicity monitoring in locations remote to the treatment process. It has been demonstrated that wastewater toxicity monitoring can be achieved by measuring the gaseous response of a biofilm, grown and sustained under high loading rates and fed continuously with the medium which to be measured.

Several areas of future research have been identified.

7.1 Expanding toxic shock testing

The response of the system to high concentrations of heavy metals known to be toxic to nitrification was confirmed. However, the response of the system to biocidal substances such as cyanide and volatile organics was not explored in this EngD project due to a lack of safe facilities in place to handle such chemicals. It would be beneficial to explore the EWS's response to these toxicants, with the condition that safe systems of work are put in place.

The likely response to such a chemical would be a complete drop in CO_2 and N_2O emissions along with a rise in DO, representing a loss of metabolic activity.

7.2 Monitoring methane as a stress response

During this study, appraisals were made on the likely origin of emissions from a literature study to understand the inhibitory mechanisms. Emissions of N_2O and CO_2 gave an indication of nitrification, denitrification and AH activity and it was deduced that methanogenesis would also likely contribute significantly to the CO_2 emissions during toxic shock.

Methanogens are known to be sensitive to heavy metal toxicity (Capone et al., 1983; Sanchez et al., 1996; Tchobanoglous et al., 2014b). The response to toxicity was measureable through a drop in the conversion rate of CO_2 to CH_4 in step 8 of the nitrogen and carbon transformation pathway (Figure 1.1).

However, to fully verify the role of methanogens, and confirm their presence in the anaerobic layers of the CFBBR biofilm, CH_4 should be added to the suite of gases monitored. At the point of a toxic shock, an opposing dip in CH_4 emissions is expected against a rise in N_2O and CO_2.

7.3 Full-scale implementation of the EWS

The CFBBR system developed in this study should be deployed at various locations across a sewer network to test as part of a full-scale implementation test. The same methodology adopted in this study should be taken, paying particular attention to biofilm community development, toxicity response rationalisation, and toxicity response amplification.

7.3.1 Biofilm community development

A CFBBR biofilm culture exposed to a sewer environment will differ from the biofilm tested in this study with respect to the sewage composition it is exposed to. The biofilm response does not need to be identical or even comparable to this study, but does need to be representative of the secondary treatment process at the WwTW the EWS is protecting. To ensure this is the case, the same approach employed in this study, whereby biofilm is developed over long a time period in a growth reactor R_0 located at the secondary treatment process should be taken. This will ensure a level of consistency of biofilm community structures deployed to the various monitoring locations in the sewer network.

However, over long term operation the community of the biofilm in the sewer network would alter, potentially to different degrees at the various deployment locations. It is likely that the sewage composition will differ across the various locations leading to different community structures over long term operation. Therefore, careful validation of the toxicity response is required.

7.3.2 Toxicity response rationalisation

It is important to compare the biofilm toxicity response at the various deployment locations, to reduce the risk of false negative / positive responses. The same methodology used in this study for response validation (described in section 4.5.3) should be translated into the full scale implementation scheme. Dose response tests should be conducted regularly for the in-network CFBBR biofilms, and compared against each other and the secondary treatment culture at the WwTW.

The ability to carry out toxic shock testing at each location would also be beneficial. This would be possible by employing more than one CFBBR at each location. The methodology would be as follows:

- A duty CFBBR would be selected, and only used for continuous monitoring of the crude sewage. Long term operation would allow a profile of the baseline conditions at the various monitoring location to be built, and compared against the toxicity response.
- The other CFBBR's would effectively be test reactors, whereby the gaseous response of the biofilm to a toxicant is monitored at set intervals using the same methodology described in section 4.4.2.
- By comparing the response at each location, an accurate profile of the variation could be built. It would also allow greater confidence and understanding of the EWS's capability.

7.3.3 Toxicity response amplification

In this study, the volume of the CFBBR vessel was constrained by the available pumps for bench scale testing. To achieve a 10 minute HRT (essential for quick toxicity detection) with the available pumping equipment, the reactor volume was limited to 7 litres. This low HRT led to high loading rates, subsequently resulting in low nitrification rates (discussed in section 4.6.4). It is possible that this low HRT in the CFBBR systems led to lower sensitivity of the biofilm to heavy metal toxicity (particularly for Cr^{6+} and Ni^{2+}).

The sensitivity of the biofilm itself cannot be increased, nor can the nitrification rate. However, increasing the available biofilm growth surface will allow amplification of the response. The larger culture size would emit a higher mass of N_2O and CO_2 into the headspace. By maintaining the same headspace volume employed on the 7 litre CFBBRs in this study, the concentration of N_2O and CO_2 would increase, effectively increasing the systems sensitivity to lower levels of toxicity.

This approach would require a larger CFBBR volume to permit a large quantity of biofilm carriers, as the solids hold up in the reactors cannot exceed 14 %v/v (discussed

in section 4.3.2). Therefore it would also require a larger feed pump to maintain an HRT of 10 minutes.

7.3.4 Post shock biofilm re-seed

Biofilms are known to adapt to toxicity after exposure (Koechler et al., 2015). Following a toxic shock, it is possible that the biofilm will recover with increased resistance to the relevant toxicant. Therefore, it is imperative that following a toxic shock the biofilm at all monitoring locations is replaced with biofilm from reactor R_0. This will ensure a degree of uniformity across all biofilms at the various monitoring locations in the network. As such, the original biofilm should be disposed of.

7.4 The role of an EWS in real time control

The role of an EWS could extend past toxicity monitoring into the realm of process analytical technology. RTC and automation of urban wastewater systems has been gaining interest in recent years and involves smart operation of infrastructure to meet tight discharge consents and improve energy efficiency (Lacour and Schütze, 2011; Langeveld et al., 2013; Schütze and Muschalla, 2013). At the WwTW, RTC involves continuous monitoring and recording of operational parameters such as air flow rates, sludge age and energy consumption, to allow proactive adjustment of treatment performance at the ASP (Schütze et al., 2004). In the sewer network, RTC generally focusses on pro-active management of SPS's, maximising the use of in-sewer storage capacity to adjust the flow and indeed load to the works (Seggelke et al., 2013). As previously highlighted, fluctuations in volume and wastewater composition at the inlet works relate directly back to the sewer (Langeveld et al., 2002), as such, extending the RTC to include in-sewer predictions of load could improve WwTW performance and energy efficiency. Limitations in the available equipment for in-sewer RTC implementation are related to the harsh environment of crude, unscreened sewage (Pedersen and Petersen, 1996). Therefore, many employ sensor cleaning and sample pre-treatment systems which invariably increase the cost of in-sewer sensors (Campisano et al., 2013). The non-contact method of headspace gas analysis could help to address this, and reduce the cost of in-sewer RTC.

It was demonstrated in this study that daily N_2O and CO_2 emissions patterns are observed from the CFBBR (Figure 4.16). These fluctuations likely resulted from changing nitrogen and organic loads in the wastewater, either during peak load periods, or as a result of flow / level conditions. As such, if links can be made between emissions and load, the biofilm based EWS proposed in this study has the potential to be implemented in an RTC system to proactively manage or balance load to the WwTW.

REFERENCES

AA. (2009), “Fuel Price Report October 2009”, available at: http://www.theaa.com/onlinenews/allaboutcars/fuel/2009/october2009.pdf (accessed 16 November 2014).

Aboobakar, A., Cartmell, E., Stephenson, T., Jones, M., Vale, P. and Dotro, G. (2013), “Nitrous oxide emissions and dissolved oxygen profiling in a full-scale nitrifying activated sludge treatment plant.”, *Water Research*, Vol. 47 No. 2, pp. 524–34.

Ahyerre, M., Chebbo, G. and Saad, M. (2001), “Nature and dynamics of water sediment interfaced in combined sewers”, *Journal of Environmental Engineering*, Vol. 127 No. 3, pp. 233–239.

Amann, R., Ludwig, W. and Schleifer, K. (1995), “Phylogenetic identification and in situ detection of individual microbial cells without cultivation”, *Microbiological Reviews*, Vol. 59 No. 1, pp. 143–169.

Amann, R., Snaidr, J., Wagner, M., Ludwig, W. and Schleifer, K. (1996), “In situ visualization of high genetic diversity in a natural microbial community”, *Journal of Bacteriology*, Vol. 178 No. 12, pp. 3496–3500.

Andreottola, G., Foladori, P. and Ragazzi, M. (2000), “Upgrading of a small wastewater treatment plant in a cold climate region using a moving bed biofilm reactor (MBBR) system”, *Water Science and Technology*, Vol. 41 No. 1, pp. 177–185.

APHA. (2005), *Standard Methods for the Examination of Water and Wastewater*, 21st ed., APHA, AWWA and WEF, Washington D.C. USA.

AppliTek. (2010), “Water Quality”, available at: www.applitek.com/en/offer/analyzers/water-quality (accessed 20 August 2011).

App-Tek. (2011), “RTx Brochure”, available at: www.odalog.com/odalog/ (accessed 19 September 2011).

Ashley, R.M., Fraser, A., Burrows, R. and Blanksby, J. (2000), “The management of sediment in combined sewers”, *Urban Water*, Vol. 2 No. 4, pp. 263–275.

Aulenta, F., Bassani, C., Ligthart, J., Majone, M. and Tilche, A. (2002), “Calorimetry: a tool for assessing microbial activity under aerobic and anoxic conditions”, *Water Research*, Vol. 36 No. 5, pp. 1297–1305.

Avery, S., Howlett, N. and Radice, S. (1996), “Copper toxicity towards Saccharomyces cerevisiae: Dependence on plasma membrane fatty acid composition”, *Applied and Environmental Microbiology*, Vol. 62 No. 11, pp. 3960–3966.

Baban, A. and Talinli, I. (2009), “Modeling of organic matter removal and nitrification in sewer systems — an approach to wastewater treatment”, *Desalination*, Vol. 246 No. 1-3, pp. 640–647.

Banasiak, R., Verhoeven, R., De Sutter, R. and Tait, S. (2005), “The erosion behaviour of biologically active sewer sediment deposits: observations from a laboratory study.”, *Water Research*, Vol. 39 No. 20, pp. 5221–31.

Bank of England. (2014), “Inflation Report August 2014”, available at: http://www.bankofengland.co.uk/publications/Pages/inflationreport/2014/ir1403.as

px (accessed 4 September 2014).

Barber, W. and Stuckey, D. (2000), "Nitrogen removal in a modified anaerobic baffled reactor (ABR): 2, nitrification", *Water Research*, Vol. 34 No. 9, pp. 2423–2432.

Bassin, J., Kleerebezem, R., Rosado, A., Van Loosdrecht, M. and Dezotti, M. (2012), "Effect of different operational conditions on biofilm development, nitrification, and nitrifying microbial population in moving-bed biofilm reactors", *Environmental Science & Technology*, Vol. 46 No. 3, pp. 1546–1555.

BBC News. (2014), "River Trent pollution charges dropped against waste firm", available at: http://www.bbc.co.uk/news/uk-england-stoke-staffordshire-27783266 (accessed 4 September 2014).

Belkin, S. (2003), "Microbial whole-cell sensing systems of environmental pollutants", *Current Opinion in Microbiology*, Vol. 6 No. 3, pp. 206–212.

Berg, I. (2011), "Ecological aspects of the distribution of different autotrophic CO2 fixation pathways", *Applied and Environmental Microbiology*, Vol. 77 No. 6, pp. 1925–1936.

Beyenal, N.Y., Özbelge-Baser, T.A. and Özbelge, H.Ö. (1997), "Combined effects of Cu2+ and Zn2+ on activated sludge process", *Water Research*, Vol. 31 No. 4, pp. 699–704.

Black, G., Jones, M., Vale, P., Johnson, N., Nocker, A., Cartmell, E. and Dotro, G. (2014), "Biofilm Responses to Toxic Shocks in Closed Pipes: Using Nitrous Oxide Emissions as an Early Warning of Toxicity Ahead of a Wastewater Treatment Works", *Water, Air and Soil Pollution*, Vol. 225 No. 2, p. 1837.

Bock Gu, M. and Cheol Gil, G. (2001), "A multi-channel continuous toxicity monitoring system using recombinant bioluminescent bacteria for classification of toxicity", *Biosensors and Bioelectronics*, Vol. 16 No. 9-12, pp. 661–666.

Bond, P., Erhart, R., Wagner, M., Keller, J. and Blackall, L. (1999), "Identification of some of the major groups of bacteria in efficient and nonefficient biological phosphorus removal activated sludge systems", *Applied and Environmental Microbiology*, Vol. 65 No. 9, pp. 4077–4084.

Bourgeois, W., Burgess, J. and Stuetz, R. (2001), "On-line monitoring of wastewater quality: A review", *Journal of Chemical Technology and Biotechnology*, Vol. 76 No. 4, pp. 337–348.

Bourgeois, W. and Stuetz, R. (2000), "Measuring wastewater quality using a sensor array: Prospects for real-time monitoring", *Water Science and Technology*, Vol. 41 No. 12, pp. 107–112.

Bourgeois, W. and Stuetz, R.. (2002), "Use of a chemical sensor array for detecting pollutants in domestic wastewater", *Water Research*, Vol. 36 No. 18, pp. 4505–4512.

Bowker, R.P.G., Smith, J.M., Shah, H.J. and Flaherty, P.A. (1991), *Hydrogen Sulfide Corrosion in Wastewater Collection and Treatment Systems, 430/09-91-010*, Washington D.C. USA.

Burgess, J., Colliver, B., Stuetz, R. and Stephenson, T. (2002), "Dinitrogen oxide

production by a mixed culture of nitrifying bacteria during ammonia shock loading and aeration failure", *Journal of Industrial Microbiology and Biotechnology*, Vol. 29 No. 6, pp. 309–313.

Burgess, J., Stuetz, R., Morton, S. and Stephenson, T. (2002), "Dinitrogen oxide detection for process failure early warning systems", *Water Science and Technology*, Vol. 45 No. 4-5, pp. 247–254.

Burton Mail. (2014), "Firm cleared over River Trent pollution charges", available at: http://www.burtonmail.co.uk/Firm-cleared-River-Trent-pollution-charges/story-21520422-detail/story.html (accessed 4 September 2014).

Butler, M., Stephenson, T., Strokes, L. and Stuetz, R. (2005), "Dinitrogen oxide detection for nitrification failure early warning systems", *Water Science and Technology*, Vol. 52 No. 8, pp. 249–256.

Butler, M.D., Wang, Y.Y., Cartmell, E. and Stephenson, T. (2009), "Nitrous oxide emissions for early warning of biological nitrification failure in activated sludge.", *Water Research*, Vol. 43 No. 5, pp. 1265–72.

Cabrero, A., Fernandez, S., Mirada, F. and Garcia, J. (1998), "Effects of copper and zinc on the activated sludge bacteria growth kinetics", *Water Research*, Vol. 32 No. 5, pp. 1355–1362.

Callister, S., Stephenson, T., Butler, M. and Cartmell, E. (2006), "N-Tox® - Early warning of nitrification toxicity for activated sludge treatment", *4th CIWEM Annual Conference*, Newcastle, UK.

Campisano, A., Cabot Ple, J., Muschalla, D., Pleau, M. and Vanrolleghem, P. (2013), "Potential and limitations of modern equipment for real time control of urban wastewater systems", *Urban Water Journal*, Vol. 10 No. 5, pp. 300–311.

Capone, D., Reese, D. and Kiene, R. (1983), "Effects of metals on methanogenesis, sulfate reduction, carbon dioxide evolution, and microbial biomass in anoxic salt marsh sediments", *Applied and Environmental Microbiology*, Vol. 45 No. 5, pp. 1586–1591.

Ceçen, F., Semerci, N. and Geyik, A.G. (2010), "Inhibition of respiration and distribution of Cd, Pb, Hg, Ag and Cr species in a nitrifying sludge.", *Journal of Hazardous Materials*, Vol. 178 No. 1-3, pp. 619–27.

Challenge Technology. (2001), "ODM-100 Brochure", available at: www.challenge-sys.com/files/ODM_New_Brochure10-11.pdf (accessed 13 May 2011).

Chandran, K. and Smets, B. (2000), "Applicability of two-step models in estimating nitrification kinetics from batch respirograms under different relative dynamics of ammonia and nitrite oxidation", *Biotechnology and Bioengineering*, Vol. 70 No. 1, pp. 54–64.

Chen, G.-H., Leung, D.H.W. and Hung, J.-C. (2003), "Biofilm in the sediment phase of a sanitary gravity sewer.", *Water Research*, Vol. 37 No. 11, pp. 2784–8.

Choung, Y.-K. and Kim, H.-S. (2000), "Nutrient Removal and ORP Monitoring in the Intermittent Aeration System with Continuous Inflow", *Environmental Technology*, Vol. 21 No. 4, pp. 375–385.

Clemens, J. and Haas, B. (1997), "Nitrous oxide emissions in sewer systems", *Acta Hydrochimica et Hydrobiologica*, Vol. 25 No. 2, pp. 96–99.

Cogent Environmental. (2011), "Online Monitoring", available at: www.cogentenvironmental.co.uk/ova.php (accessed 15 August 2011).

Cokgor, E.U., Ozdemir, S., Karahan, O., Insel, G. and Orhon, D. (2007), "Critical appraisal of respirometric methods for metal inhibition on activated sludge.", *Journal of Hazardous Materials*, Vol. 139 No. 2, pp. 332–9.

Colliver, B.B. and Stephenson, T. (2000), "Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers", *Biotechnology Advances*, Vol. 18 No. 3, pp. 219–232.

Cui, Y., Liu, H. and Bai, C. (2008), "Circulating fluidized bed biological reactor for nutrients removal", *Frontiers of Environmental Science and Engineering in China*, Vol. 2 No. 3, pp. 349–353.

Daims, H., Nielsen, J., Nielsen, P., Schleifer, K. and Wagner, M. (2001), "In Situ Characterization of Nitrospira-Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants", *Applied and Environmental Microbiology*, Vol. 67 No. 3-12, pp. 5273–5284.

Debruyn, W., Wevers, M. and van Rensbergen, J. (1994), "The measurement of nitrous oxide emissions from sewage systems in Belgium", *Fertilizer Research*, Vol. 37 No. 3, pp. 201–205.

Demirel, B., Yenigun, O. and Onay, T.T. (2005), "Anaerobic treatment of dairy wastewaters: a review", *Process Biochemistry*, Vol. 40 No. 8, pp. 2583–2595.

Desloover, J., Vlaeminck, S.E., Clauwaert, P., Verstraete, W. and Boon, N. (2012), "Strategies to mitigate N2O emissions from biological nitrogen removal systems.", *Current Opinion in Biotechnology*, Vol. 23 No. 3, pp. 474–82.

Dewettinck, T., Van Hege, K. and Verstraete, W. (2001), "The electronic nose as a rapid sensor for volatile compounds in treated domestic wastewater", *Water Research*, Vol. 35 No. 10, pp. 2475–2483.

Direct Water Tanks. (2014), "50 Litre Water Tank", available at: http://www.directwatertanks.co.uk/water-tanks/50-litre-water-tank (accessed 10 September 2014).

Dotro, G. (2009), "Early Warning and Detection of Potentially Toxic Substances for Pollution Incident Prevention and Control", Severn Trent Water, Coventry, UK.

Dotro, G., Jefferson, B., Jones, M., Vale, P., Cartmell, E. and Stephenson, T. (2011), "A review of the impact and potential of intermittent aeration on continuous flow nitrifying activated sludge", *Environmental Technology*, Vol. 32 No. 15, pp. 1685–1697.

Dulkadiroglu, H., Cokgor, E., Artan, N. and Orhon, D. (2005), "The effect of temperature and sludge age on COD removal and nitrification in a moving bed sequencing batch biofilm reactor", *Water Science and Technology*, Vol. 51 No. 11, pp. 95–103.

edie. (1999), "Yorkshire water cleared in river Calder cyanide fish kil", available at:

www.edie.net/news/news_story.asp?id=1714&channel=3&title=Yorkshire+Water+cleared+in +River+Calder+cyanide+ fish+kill (accessed 1 October 2011).

edie. (2000), "US sues companies for massive fish kill in Indiana", available at: www.edie.net/news/news_story.asp?id=2665&channel=0&title=US+sues+companies+for+ma ssive+fish+kill+in+India (accessed 1 October 2011).

Eldyasti, A., Andalib, M., Hafez, H., Nakhla, G. and Zhu, J. (2011), "Comparative modeling of biological nutrient removal from landfill leachate using a circulating fluidized bed bioreactor (CFBBR).", *Journal of Hazardous Materials*, Vol. 187 No. 1-3, pp. 140–9.

Environment Agency. (2009), "Courts urged to issue higher fines for environmental pollution", available at: http://webarchive.nationalarchives.gov.uk/20101012203020/http://www.environment-agency.gov.uk/cy/new/122966.aspx?month=11&year=2009 (accessed 4 September 2014).

Envitech. (2005a), "WWTP toxicity detection applications", available at: www.envitech.co.uk/Articles_Toxicity.html (accessed 30 August 2011).

Envitech. (2005b), "Nitrification - WWTP process control & monitoring", available at: www.envitech.co.uk/Articles_Nitrification.html (accessed 30 August 2011).

Farré, M. and Barceló, D. (2003), "Toxicity testing of wastewater and sewage sludge by biosensors, bioassays and chemical analysis", *TrAC Trends in Analytical Chemistry*, Vol. 22 No. 5, pp. 299–310.

Fisher Scientific. (2014), "12446350 - Peristaltic pump 520S/R 158mm x 276mm manual", available at: https://webshop.fishersci.com/insight2_uk/getProduct.do?productCode=12446350&resultSetPosition=0 (accessed 10 September 2014).

Forster, S., Lappin-Scott, H.M., Snape, J.R. and Porter, J. (2003), "Rains, drains and active strains: towards online assessment of wastewater bacterial communities", *Journal of Microbiological Methods*, Vol. 55 No. 3, pp. 859–864.

Geenens, D. and Thoeye, C. (1998), "The use of an on-line respirometer for the screening of toxicity in the antwerp WWTP catchment area", *Water Science and Technology*, Vol. 37 No. 12, pp. 213–218.

Gerardi, M.H. (2002), *Nitrification and Denitrification in the Activated Sludge Process*, First., John Wiley and Sons, New York.

Gernaey, K., Bogaert, H., Massone, A., Vanrolleghem, P. and Verstraete, W. (1997), "On-line nitrification monitoring in activated sludge with a titrimetric sensor", *Environmental Science & Technology*, Vol. 31 No. 8, pp. 2350–2355.

Gieseke, A., Purkhold, U., Wagner, M., Amann, R. and Schramm, A. (2001), "Community Structure and Activity Dynamics of Nitrifying Bacteria in a Phosphate-Removing Biofilm", *Applied and Environmental Microbiology*, Vol. 67 No. 3, pp. 1351–1362.

Gikas, P. (2008), "Single and combined effects of nickel (Ni(II)) and cobalt (Co(II)) ions on activated sludge and on other aerobic microorganisms: a review.", *Journal of Hazardous Materials*, Vol. 159 No. 2-3, pp. 187–203.

Giller, K.E., Witter, E. and Mcgrath, S.P. (1998), "Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review", *Soil Biology and Biochemistry*, Vol. 30 No. 10-11, pp. 1389–1414.

GOV.UK. (2014), "Prices of fuels purchased by manufacturing industry", available at: https://www.gov.uk/government/statistical-data-sets/prices-of-fuels-purchased-by-manufacturing-industry (accessed 4 September 2014).

Gromaire, M.., Garnaud, S., Saad, M. and Chebbo, G. (2001), "Contribution of different sources to the pollution of wet weather flows in combined sewers", *Water Research*, Vol. 35 No. 2, pp. 521–533.

Gutiérrez, M., Etxebarria, J. and de las Fuentes, L. (2002), "Evaluation of wastewater toxicity: comparative study between Microtox® and activated sludge oxygen uptake inhibition", *Water Research*, Vol. 36 No. 4, pp. 919–924.

H3G. (2014), "ZTE MF823 4G Dongle", available at: http://www.three.co.uk/Discover/Devices/ZTE/MF823_4G_Dongle?memory=0&colour=Black (accessed 10 September 2014).

Hayes, E., Upton, J., Batts, R. and Pickin, S. (1998), "On-line nitrification inhibition monitoring using immobilised bacteria", *Water Science and Technology*, Vol. 37 No. 12, pp. 193–196.

Hem, L.J., Rusten, B. and Ødegaard, H. (1994), "Nitrification in a moving bed biofilm reactor", *Water Research*, Vol. 28 No. 6, pp. 1425–1433.

HFL Risk. (2011), "No fine for cyanide accident firm", available at: http://www.hflrisk.com/news-item/800359519 (accessed 4 September 2014).

Hoque, M., Aravinthan, V., Pradhan, N. and Sakhiya, A. (2011), "Combined titrimetric respirometer as a real-time sensor to monitor the aerobic biodegradation process of different substrates", *Journal of Applied Sciences*, Vol. 11 No. 13, pp. 2299–2305.

Houhou, J., Lartiges, B.S., Montarges-Pelletier, E., Sieliechi, J., Ghanbaja, J. and Kohler, A. (2009), "Sources, nature, and fate of heavy metal-bearing particles in the sewer system.", *The Science of the Total Environment*, Vol. 407 No. 23, pp. 6052–62.

Hu, Z., Chandran, K., Grasso, D. and Smets, B. (2003), "Impact of metal sorption and internalization on nitrification inhibition", *Environmental Science and Technology*, Vol. 37 No. 4, pp. 728–734.

Hu, Z., Chandran, K., Grasso, D. and Smets, B.F. (2004), "Comparison of nitrification inhibition by metals in batch and continuous flow reactors.", *Water Research*, Vol. 38 No. 18, pp. 3949–59.

Huddersfield Examiner. (2011), "Chemical company Syngenta avoids fine for cyanide accident", available at: http://www.examiner.co.uk/news/west-yorkshire-news/chemical-company-syngenta-avoids-fine-4983636 (accessed 4 September 2014).

International Standards Organisation. (1989), "Water Quality – Determination of the chemical oxygen demand. ISO Method 6060."

International Standards Organisation. (1999), "Water Quality – Guidelines for the

determination of total organic carbon (TOC) and dissolved organic carbon (DOC). ISO Method 8245."

International Standards Organisation. (2003), "Water Quality – Determination of biochemical oxygen demand after n days (BODn) – Part 1: Dilution and seeding method with allythiourea addition. ISO Method 5815-1."

International Standards Organisation. (2006), "Water Quality – Toxicity testing for assessing the inhibition of nitrification of activated sludge microorganisms. ISO Method 9509."

International Standards Organisation. (2007), "Water quality – test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation. IS0 Method 8192."

International Standards Organisation. (2008a), "Water Quality – Determination of the dissolved oxygen – Electrochemical probe method. ISO Method 5814."

International Standards Organisation. (2008b), "Water quality – Determination of pH. ISO Method 10523."

Jiang, F., Leung, D.H.-W., Li, S., Chen, G.-H., Okabe, S. and van Loosdrecht, M.C.M. (2009), "A biofilm model for prediction of pollutant transformation in sewers.", *Water Research*, Vol. 43 No. 13, pp. 3187–98.

Jiang, G., Gutierrez, O. and Yuan, Z. (2011), "The strong biocidal effect of free nitrous acid on anaerobic sewer biofilms.", *Water Research*, Vol. 45 No. 12, pp. 3735–43.

Jönsson, K. (2000), "Occurrence of nitrification inhibition in Swedish municipal wastewaters", *Water Research*, Vol. 34 No. 9, pp. 2455–2462.

Jönsson, K., Aspichueta, E., De La Sota, A. and Jansen, J. (2001), "Evaluation of nitrification-inhibition measurements", *Water Science and Technology*, Vol. 43 No. 1, pp. 201–208.

Kampschreur, M., Tan, N., Kleerebezem, R., Picioreanu, C., Jetten, M. and Van Loosdrecht, M. (2008), "Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture", *Environmental Science and Technology*, Vol. 42 No. 2, pp. 429–435.

Kampschreur, M.J., van der Star, W.R.L., Wielders, H.A., Mulder, J.W., Jetten, M.S.M. and van Loosdrecht, M.C.M. (2008), "Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment.", *Water Research*, Vol. 42 No. 3, pp. 812–26.

Karunakaran, E., Mukherjee, J., Ramalingan, B. and Biggs, C.A. (2011), "' Biofilmology ' : a multidisciplinary review of the study of microbial biofilms", *Applied Microbiology and Biotechnology*, Vol. 90, pp. 1869–1881.

Kelma. (2011), "RODTOX 2000", available at: www.kelma.com/product/71/rodtox-2000 (accessed 16 August 2011).

Kim, S.-W., Miyahara, M., Fushinobu, S., Wakagi, T. and Shoun, H. (2010), "Nitrous oxide emission from nitrifying activated sludge dependent on denitrification by ammonia-oxidizing bacteria.", *Bioresource Technology*, Vol. 101 No. 11, pp. 3958–63.

Kiptoo, J.K., Ngila, J.C. and Sawula, G.M. (2004), "Speciation studies of nickel and chromium in wastewater from an electroplating plant.", *Talanta*, Vol. 64 No. 1, pp. 54–9.

Koechler, S., Farasin, J., Cleiss-Arnold, J. and Arsène-Ploetze, F. (2015), "Toxic metal resistance in biofilms: diversity of microbial responses and their evolution", *Research in Microbiology*, Vol. 166 No. 10, pp. 764–773.

Kong, Z., Vanrolleghem, P.A. and Verstraete, W. (1993), "An activated sludge-based biosensor for rapid IC50 estimation and on-line toxicity monitoring", *Biosensors and Bioelectronics*, Vol. 8 No. 1, pp. 49–58.

König, A., Riedel, K. and Metzger, J.W. (1998), "A microbial sensor for detecting inhibitors of nitrification in wastewater", *Biosensors and Bioelectronics*, Vol. 13 No. 7-8, pp. 869–874.

Kroiss, H., Schweighofer, P., Frey, W. and Matsche, N. (1992), "Nitrification inhibition - A source identification method for combined municipal and/or industrial wastewater treatment plants", *Water Science and Technology*, Vol. 26 No. 5-6, pp. 1135–1146.

Krul, J. and Veeningen, R. (1977), "The synthesis of the dissimilatory nitrate reductase under aerobic conditions in a number of denitrifying bacteria, isolated from activated sludge and drinking water", *Water Research*, Vol. 11 No. 1, pp. 39–43.

Lacour, C. and Schütze, M. (2011), "Real-time control of sewer systems using turbidity measurements", *Water Science and Technology*, Vol. 63 No. 11, pp. 2628–2632.

Lajoie, C.A., Lin, S.-C., Nguyen, H. and Kelly, C.J. (2002), "A toxicity testing protocol using a bioluminescent reporter bacterium from activated sludge", *Journal of Microbiological Methods*, Vol. 50 No. 3, pp. 273–282.

Langeveld, J., Benedetti, L., de Klein, J., Nopens, I., Amerlinck, Y., van Nieuwenhuijzen, A Flameling, T., van Zanten, O., et al. (2013), "Impact-based integrated real-time control for improvement of the Dommel River water quality", *Urban Water Journal*, Vol. 10 No. 5, pp. 312–329.

Langeveld, J.G., Clemens, F.H.L.R. and Van Der Graaf, J.H.J.M. (2002), "Increasing wastewater system performance - The importance of interactions between sewerage and wastewater treatment", *Water Science and Technology*, Vol. 45 No. 3, pp. 45–52.

LAR Process Analysers. (2016), "Toximeter NitriTox", available at: http://www.lar.com/products/bod-toxicity/nitritox.html (accessed 21 February 2016).

Larsen, P., Nielsen, J.L., Svendsen, T.C. and Nielsen, P.H. (2008), "Adhesion characteristics of nitrifying bacteria in activated sludge.", *Water Research*, Vol. 42 No. 10-11, pp. 2814–26.

Lazarova, V. and Manem, J. (1996), "An innovative process for waste water treatent: The circulating floating bed reactor", *Water Science & Technology*, Vol. 96 No. 9, pp. 89–99.

Lazarova, V., Meyniel, J., Duval, L. and Manem, J. (1997), "A novel circulating bed reactor: Hydrodynamics, mass transfer and nitrification capacity", *Chemical*

Engineering Science, Vol. 52 No. 21-22, pp. 3919–3927.

Lazarova, V., Nogueira, R., Manem, J. and Melo, L. (1998), "Influence of dissolved oxygen on nitrification kinetics in a circulating bed reactor", *Water Science and Technology*, Vol. 37 No. 4-5, pp. 189–193.

Lee, Y., Tian, Q., Ong, S., Sato, C. and Chung, J. (2009), "Inhibitory Effects of Copper on Nitrifying Bacteria in Suspended and Attached Growth Reactors", *Water, Air, & Soil Pollution*, Vol. 203 No. 1, pp. 17–27.

Lei, Y., Chen, W. and Mulchandani, A. (2006), "Microbial biosensors", *Analytica Chimica Acta*, Vol. 568 No. 1-2, pp. 200–210.

Leu, S.-Y., Libra, J.A. and Stenstrom, M.K. (2010), "Monitoring off-gas O2/CO2 to predict nitrification performance in activated sludge processes.", *Water Research*, Vol. 44 No. 11, pp. 3434–44.

Li, M., Nakhla, G. and Zhu, J. (2012), "Simultaneous carbon and nitrogen removal with enhanced bioparticle circulation in a Circulating Fluidized Bed Biofilm Reactor", *Chemical Engineering Journal*, Vol. 181-182, pp. 35–44.

Lo, I.W., Lo, K.V., Mavinic, D.S., Shiskowski, D. and Ramey, W. (2010), "Contributions of biofilm and suspended sludge to nitrogen transformation and nitrous oxide emission in hybrid sequencing batch system", *Journal of Environmental Sciences*, Vol. 22 No. 7, pp. 953–960.

Love, N.G. and Bott, C.B. (2000), *A Review and Needs Survey of Upset Early Warning Devices*, Water Environment Research Foundation, Alxandria, VA, USA.

Machine Mart. (2014), "Clarke Wiz Mini Air Compressor", available at: https://www.machinemart.co.uk/p/wiz-mini-air-compressor/ (accessed 10 September 2014).

Madoni, P., Davoli, D. and Guglielmi, L. (1999), "Response of sOUR and AUR to heavy metal contamination in activated sludge", *Water Research*, Vol. 33 No. 10, pp. 2459–2464.

Martell, A. (1981), "Chemistry of carcinogenic metals", *Environmental Health Perspectives*, Vol. 40, pp. 207–226.

Martín-Cereceda, M., Pérez-Uz, B., Serrano, S. and Guinea, A. (2002), "An integrated approach to analyse biofilms of a full scale wastewater treatment plant", *Water Science and Technology*, Vol. 46 No. 1-2, pp. 199–206.

Mazierski, J. (1994), "Effect of chromium (CrVI) on the growth rate of denitrifying bacteria", *Water Research*, Vol. 28 No. 9, pp. 1981–1985.

McCarty, G.W. (1999), "Modes of action of nitrification inhibitors", *Biology and Fertility of Soils*, Vol. 29 No. 1, pp. 1–9.

Modern Water. (2016), "Online Toxicity Monitoring", available at: http://www.modernwater.com/monitoring/toxicity/on-line-microtox-ctm (accessed 21 February 2016).

Multisensor Systems. (2012), "MS1100 VOC hydrocarbon event monitor, oil in water monitor", available at: www.multisensor.co.uk/product.asp/id/5/vocms 1100.htm (accessed 30 January 2012).

Newton, J. and Reid, S. (2007), "The role and application of cost benefit analysis, 07/RG/07", K Water Industry Research Limited, London.

Nielsen, P., Raunkjaer, K., Norsker, N., Jensen, N. and Hvitved-Jacobsen, T. (1992), "Transformation of wastewater in sewer systems - A review", *Water Science and Technology*, Vol. 25 No. 6, pp. 17–31.

Nogueira, R., Melo, L.F., Purkhold, U., Wuertz, S. and Wagner, M. (2002), "Nitrifying and heterotrophic population dynamics in biofilm reactors: effects of hydraulic retention time and the presence of organic carbon", *Water Research*, Vol. 36 No. 2, pp. 469–481.

Ochoa-Herrera, V., León, G., Banihani, Q., Field, J.A. and Sierra-Alvarez, R. (2011), "Toxicity of copper(II) ions to microorganisms in biological wastewater treatment systems.", *The Science of the Total Environment*, Vol. 412-413, pp. 380–5.

Ødegaard, H. (2006), "Innovations in wastewater treatment: The moving bed biofilm process", *Water Science and Technology*, Vol. 53 No. 9, pp. 17–33.

Office for National Statistics. (2014), "Statistical Bulletin; Consumer Price Inflation August 2014", available at: http://www.ons.gov.uk/ons/rel/cpi/consumer-price-indices/august-2014/stb---consumer-price-indices---august-2014.html (accessed 4 September 2014).

OFWAT. (2015a), "Outcome delivery incentive rates for Thames Water following recalibration based on the company's menu choice", available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenutms.pdf (accessed 14 September 2015).

OFWAT. (2015b), "Outcome delivery incentive rates for Severn Trent Water following recalibration based on the company's menu choice", available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenusvt.pdf (accessed 14 September 2015).

OFWAT. (2015c), "Outcome delivery incentive rates for Anglian Water following recalibration based on the company's menu choice", available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenuanh.pdf (accessed 14 September 2015).

OFWAT. (2015d), "Outcome delivery incentive rates for Yorkshire Water following recalibration based on the company ' s menu choice", available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenuyky.pdf (accessed 14 September 2015).

OFWAT. (2015e), "Outcome delivery incentive rates for United Utilities following recalibration based on the company's menu choice", available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenuuu.pdf (accessed 14 September 2015).

OFWAT. (2015f), “Outcome delivery incentive rates for Northumbrian Water following recalibration based on the company ’ s menu choice”, available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenunes.pdf (accessed 14 September 2014).

OFWAT. (2015g), “Outcome delivery incentive rates for South West Water following recalibration based on the company’s menu choice”, available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenuswt.pdf (accessed 14 September 2015).

OFWAT. (2015h), “Outcome delivery incentive rates for Wessex Water following recalibration”, available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenwsx.pdf (accessed 14 September 2015).

OFWAT. (2015i), “Outcome delivery incentive rates for Southern Water following recalibration based on the company’s menu choice”, available at: http://www.ofwat.gov.uk/wp-content/uploads/2015/10/pap_tec201503odimenusrn.pdf (accessed 14 September 2015).

Oguz, M.T., Robinson, K.G., Layton, A.C. and Sayler, G.S. (2006), “Volatile fatty acid impacts on nitrite oxidation and carbon dioxide fixation in activated sludge.”, *Water Research*, Vol. 40 No. 4, pp. 665–74.

Ozer, A. and Kasirga, E. (1995), “Substrate removal in long sewer lines”, *Water Science and Technology*, Vol. 31 No. 7, pp. 213–218.

Pagga, U., Bachner, J. and Strotmann, U. (2006), “Inhibition of nitrification in laboratory tests and model wastewater treatment plants.”, *Chemosphere*, Vol. 65 No. 1, pp. 1–8.

Painter, H.. (1970), “A review of literature on inorganic nitrogen metabolism in microorganisms”, *Water Research*, Vol. 4 No. 6, pp. 393–450.

Pan, S., Tay, J., He, Y. and Tay, S. (2004), “The effect of hydraulic retention time on the stability of aerobically grown microbial granules”, *Letters in Applied Microbiology*, Vol. 38 No. 2, pp. 158–163.

Pashkova, A., Svajda, K., Black, G. and Dittmeyer, R. (2009), “Automated system for spectrophotometric detection of liquid phase hydrogen peroxide for concentrations up to 5% w/w.”, *The Review of Scientific Instruments*, Vol. 80 No. 5, p. 055104.

Pastorelli, G., Andreottola, G., Canziani, R., Defrajafrangipane, E., Depascalis, F., Gurrieri, G. and Rozzi, A. (1997), “Pilot-plant experiments with moving-bed biofilm reactors”, *Water Science and Technology*, Vol. 36 No. 1, pp. 43–50.

Pedersen, F. and Petersen, G. (1996), “Variability of species sensitivity to complex mixtures”, *Water Science and Technology*, Vol. 33 No. 6, pp. 109–119.

Persaud, K., Khaffaf, S., Hobbs, P. and Sneath, R. (1996), “Assessment of conducting polymer odour sensors for agricultural malodour measurements”, *Chemical Senses*, Vol. 21 No. 5, pp. 495–505.

Pipestock. (2014), “PVC”, available at: https://www.pipestock.com/pvc (accessed 10 September 2014).

PRWeb. (2011), “Challenge Technology releases online oxygen demand monitor for wastewater treatment operations”, available at: www.prweb.com/ releases/challenge-technology/oxygen-uptake-rate/prweb8240961.htm (accessed 11 May 2011).

Pump Sales Direct. (2014), “DGO 200/2/80 automatic submersible sewage pump c/w F-type float switch and capacitor box, 230v.”, available at: http://www.pumpsalesdirect.co.uk/dgo-200-2-80-automatic-submersible-sewage-pump-c-w-f-type-float-switch-and-capacitor-box-230v.html (accessed 10 September 2014).

Quinn, B., Gagné, F. and Blaise, C. (2008), “An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, Hydra attenuata.”, *The Science of the Total Environment*, Vol. 389 No. 2-3, pp. 306–14.

Ren, S. (2004), “Assessing wastewater toxicity to activated sludge: recent research and developments.”, *Environment International*, Vol. 30 No. 8, pp. 1151–64.

Ren, S. and Frymier, P. (2003), “Comparative study of two bioassays for applications in influent wastewater toxicity monitoring”, *Journal of Environmental Engineering*, Vol. 129 No. 3, pp. 216–221.

Rensing, C. and Maier, R.M. (2003), “Issues underlying use of biosensors to measure metal bioavailability”, *Ecotoxicology and Environmental Safety*, Vol. 56 No. 1, pp. 140–147.

Ricco, G., Tomei, M.C.M.C., Ramadori, R. and Laera, G. (2004), “Toxicity assessment of common xenobiotic compounds on municipal activated sludge: comparison between respirometry and Microtox.”, *Water Research*, Vol. 38 No. 8, pp. 2103–10.

Rostron, W.M., Stuckey, D.C. and Young, A.A. (2001), “Nitrification of high strength ammonia wastewaters: comparative study of immobilisation media”, *Water Research*, Vol. 35 No. 5, pp. 1169–1178.

RS online. (2014a), “ITT Flojet Diaphragm Air Operated Positive Displacement Pump, 19L/min, 8.27bar”, available at: http://uk.rs-online.com/web/p/positive-displacement-pumps/6679116/ (accessed 10 September 2014).

RS online. (2014b), “Verderflex Peristaltic Electric Operated Positive Displacement Pump, 0.562L/min, 1bar, 12 V dc”, available at: http://uk.rs-online.com/web/p/positive-displacement-pumps/7056653/ (accessed 10 September 2014).

RS online. (2014c), “Pico Technology PicoLog 1216 Data Logger, Maximum Voltage Measurement 2.5V, USB 2.0, Mains”, available at: http://uk.rs-online.com/web/p/data-loggers/6668163/ (accessed 10 September 2014).

RS online. (2015), “Mitsubishi 7 in TFT LCD Touch-Screen HMI Display, GS21 GOT2000 Colour, 800 x 480pixels 155 x 206 x 50 mm”, available at: http://uk.rs-online.com/web/p/products/8280529/ (accessed 12 August 2015).

Rusten, B., Hellström, B., Hellström, F., Sehested, O., Skjelfoss, E. and Svendsen, B. (2000), "Pilot testing and preliminary design of moving bed biofilm reactors for nitrogen removal at the FREVAR wastewater treatment plant", *Water Science and Technology*, Vol. 41 No. 4-5, pp. 13–20.

Salanitro, J., Langston, G., Dorn, P. and Kravetz, L. (1988), "Activated sludge treatment of ethoxylate surfactants at high industrial use concentrations", *Water Science and Technology*, Vol. 20 No. 11-12, pp. 125–130.

Sanchez, J., Valle, L., Rodriguez, F., Moriñigo, M. and Borrego, J. (1996), "Inhibition of methanogenesis by several heavy metals using pure cultures", *Letters in Applied Microbiology*, Vol. 23 No. 6, pp. 439–444.

Sani, R., Peyton, B. and Brown, L. (2001), "Copper-Induced Inhibition of Growth of Desulfovibrio desulfuricans G20: Assessment of Its Toxicity and Correlation with Those of Zinc and Lead", *Applied and Environmental Microbiology*, Vol. 67 No. 10, pp. 4765–4772.

Scania AM. (2009), "P-Series Technical Data Sheet: P09401EN", available at: http://www.scania.com/images/P09401EN Scania P 360 P 400_tcm10-231604_tcm40-70460.pdf (accessed 16 November 2014).

Schütze, M., Campisano, A., Colas, H., Schilling, W. and Vanrolleghem, P.A. (2004), "Real time control of urban wastewater systems—where do we stand today?", *Journal of Hydrology*, Vol. 299 No. 3-4, pp. 335–348.

Schütze, M. and Muschalla, D. (2013), "Special Issue on Real time control of urban drainage systems", *Urban Water Journal*, Vol. 10 No. 5, pp. 291–292.

Scullion, J., Winson, M. and Matthews, R. (2007), "Inhibition and recovery in a fixed microbial film leachate treatment system subject to shock loading of copper and zinc.", *Water Research*, Vol. 41 No. 18, pp. 4129–38.

Seggelke, K., Löwe, R., Beeneken, T. and Fuchs, L. (2013), "Implementation of an integrated real-time control system of sewer system and waste water treatment plant in the city of Wilhelmshaven", *Urban Water Journal*, Vol. 10 No. 5, pp. 330–341.

Semerci, N. and Ceçen, F. (2007), "Importance of cadmium speciation in nitrification inhibition.", *Journal of Hazardous Materials*, Vol. 147 No. 1-2, pp. 503–12.

Severn Trent Water. (2009a), *STW Design Manual - Hydraulic Analysis and Design, Severn Trent Water Ltd Design Manuals*, Technical Standards Manager - Civil, Coventry, UK.

Severn Trent Water. (2009b), *STW Design Manual - Storm Water Tanks, Severn Trent Water Ltd Design Manuals*, Asset creation process design group, Coventry, UK.

Severn Trent Water. (2010), *STW Design Manual - Sewage Pumping Stations and Rising Mains, Severn Trent Water Ltd Design Manuals*, Technical Standards Manager - Civil, Coventry, UK.

Short, M.D., Daikeler, A., Peters, G.M., Mann, K., Ashbolt, N.J., Stuetz, R.M. and Peirson, W.L. (2014), "Municipal gravity sewers: An unrecognised source of nitrous oxide", *Science of the Total Environment*, Vol. 468-469, pp. 211–218.

Sin, S., Chua, H., Lo, W. and Yu, P. (2000), "Effects of trace levels of copper, chromium, and zinc ions on the performance of activated sludge", *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, Vol. 84-86 No. 487-500.

Skipworth, P.., Tait, S.. and Saul, A.. (2000), "The first foul flush in combined sewers: an investigation of the causes", *Urban Water*, Vol. 2 No. 4, pp. 317–325.

Stasinakis, A.S., Thomaidis, N.S., Mamais, D., Papanikolaou, E.C., Tsakon, A. and Lekkas, T.D. (2003), "Effects of chromium (VI) addition on the activated sludge process.", *Water Research*, Vol. 37 No. 9, pp. 2140–8.

Stein, L.Y. and Klotz, M.G. (2011), "Nitrifying and denitrifying pathways of methanotrophic bacteria", *Biochemical Society Transactions*, Vol. 39 No. 6, pp. 1826–1831.

Stuetz, R.M., Fenner, R.A. and Engin, G. (1999a), "Assessment of odours from sewage treatment works by an electronic nose, H2S analysis and olfactometry", *Water Research*, Vol. 33 No. 2, pp. 453–461.

Stuetz, R.M., Fenner, R.A. and Engin, G. (1999b), "Characterisation of wastewater using an electronic nose", *Water Research*, Vol. 33 No. 2, pp. 442–452.

Tanner, T. (2008), "Managing Biofilms", *Asian Water*, p. 23.

Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G. and Pfrang, W. (2014a), "Substrate removal in attached growth treatment process", *Wastewater Engineering: Treatment and Resource Recovery*, Fifth., McGraw Hill, New York, pp. 610–615.

Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G. and Pfrang, W. (2014b), "Introduction to microbial metabolism", *Wastewater Engineering: Treatment and Resource Recovery*, Fifth., McGraw Hill, New York, pp. 571–573.

Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G. and Pfrang, W. (2014c), "Bacterial growth, energetics and decay", *Wastewater Engineering: Treatment and Resource Recovery*, Fifth., McGraw Hill, New York, pp. 573–588.

Tchobanoglous, G., Stensel, D.H., Tsuchihashi, R., Burton, F., Abu-Orf, M., Bowden, G. and Pfrang, W. (2014d), "Suspended growth biological treatment processes", *Wastewater Engineering: Treatment and Resource Recovery*, Fifth., McGraw Hill, New York, pp. 697–940.

The Crown. (2001), *Water Industry Act 1991*, The Sationary Office Limited, Norwich, UK.

The Guardian. (2009), "Untreated sewage and cyanide kill thousands of fish in river Trent", available at: http://www.theguardian.com/uk/2009/oct/07/pollution-river-trent-investigation (accessed 4 September 2014).

The Independent. (2009), "Cyanide sparks River Trent pollution probe", available at: http://www.independent.co.uk/environment/green-living/cyanide-sparks-river-trent-pollution-probe-1798896.html (accessed 4 September 2014).

The Telegraph. (2009), “Pollution warning over raw sewage in River Trent”, available at: http://www.telegraph.co.uk/earth/environment/6266925/Pollution-warning-over-raw-sewage-in-River-Trent.html (accessed 4 September 2014).

Tyagi, V.K., Bhatia, A., Gaur, R.Z., Khan, A.A., Ali, M., Khursheed, A. and Kazmi, A.A. (2012), “Effects of multi-metal toxicity on the performance of sewage treatment system during the festival of colors (Holi) in India.”, *Environmental Monitoring and Assessment*, Vol. 184 No. 12, pp. 7517–29.

United Utilities Plc. (2008), *Secondary Activated Sludge (Incorporating Final Settlement) - Asset Standard*, *United Utilities Plc Asset Standards*, Wastewater Treatment Standards Manager, Warrington, UK.

United Utilities Plc. (2011), *Hydraulic Planning and Loading Asset Standard*, Head of Civil Engineering, Warrington, UK.

US Department of Justice. (2001), “U.S. Indiana reach environmental agreements with Guide Corp. for fish kill on the White River - Civil and criminal agreements are worth $14 million”, available at: http://www.justice.gov/opa/pr/2001/June/267enrd.html (accessed 4 September 2014).

USEPA. (2000), *Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136)*, Office of Water, Washington D.C. USA.

USEPA. (2002), *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms Fourth Edition October 2002*, Office of Water, Washington D.C. USA.

Utility Week. (2009), “Upstream cyanide pollution caused Severn Trent sewage spill - Update”, available at: www.utilityweek.co.uk/news/news_story.asp?id=70314&title=Upstream+cyanide+pollution+caused+Severn+Trent+sewage+spill+- +UPDATE (accessed 10 August 2011).

Vaiopoulou, E. and Gikas, P. (2012), “Effects of chromium on activated sludge and on the performance of wastewater treatment plants: A review.”, *Water Research*, Vol. 46 No. 3, pp. 549–70.

Vaisala. (2014), “GMD20 Duct Mounted Carbon Dioxide Transmitter for Demand Controlled Ventilation Applications”, available at: https://store.vaisala.com/uk/gmd20-duct-mounted-carbon-dioxide-transmitter-for-demand-controlled-ventilation-applications/GMD20/dp (accessed 10 September 2014).

Vanrolleghem, P., Kong, Z. and Coen, F. (1996), “Full-scale on-line assessment of toxic wastewaters causing change in biodegradation model structure and parameters”, *Water Science and Technology*, Vol. 33 No. 2, pp. 163–175.

Vanrolleghem, P. and Lee, D. (2003), “On-line monitoring equipment for wastewater treatment processes: State of the art”, *Water Science and Technology*, Vol. 47 No. 2, pp. 1–34.

Vollertsen, J., Nielsen, A., Yang, W. and Hvitved-Jacobsen, T. (2005), “Effects of in-sewer processes: A stochastic model approach”, *Water Science and Technology*,

Vol. 52 No. 3, pp. 171–180.

Water Innovate. (2011), “N-Tox”, available at: www.waterinnovate.co.uk/product-datacentre/n-tox (accessed 18 August 2011).

Weon, S., Koopman, B. and Ill Lee, S. (2004), “Effects of toxicants on nitrifying biomass in flocs vs. carriers”, *Environmental Technology*, Vol. 25 No. 2, pp. 193–199.

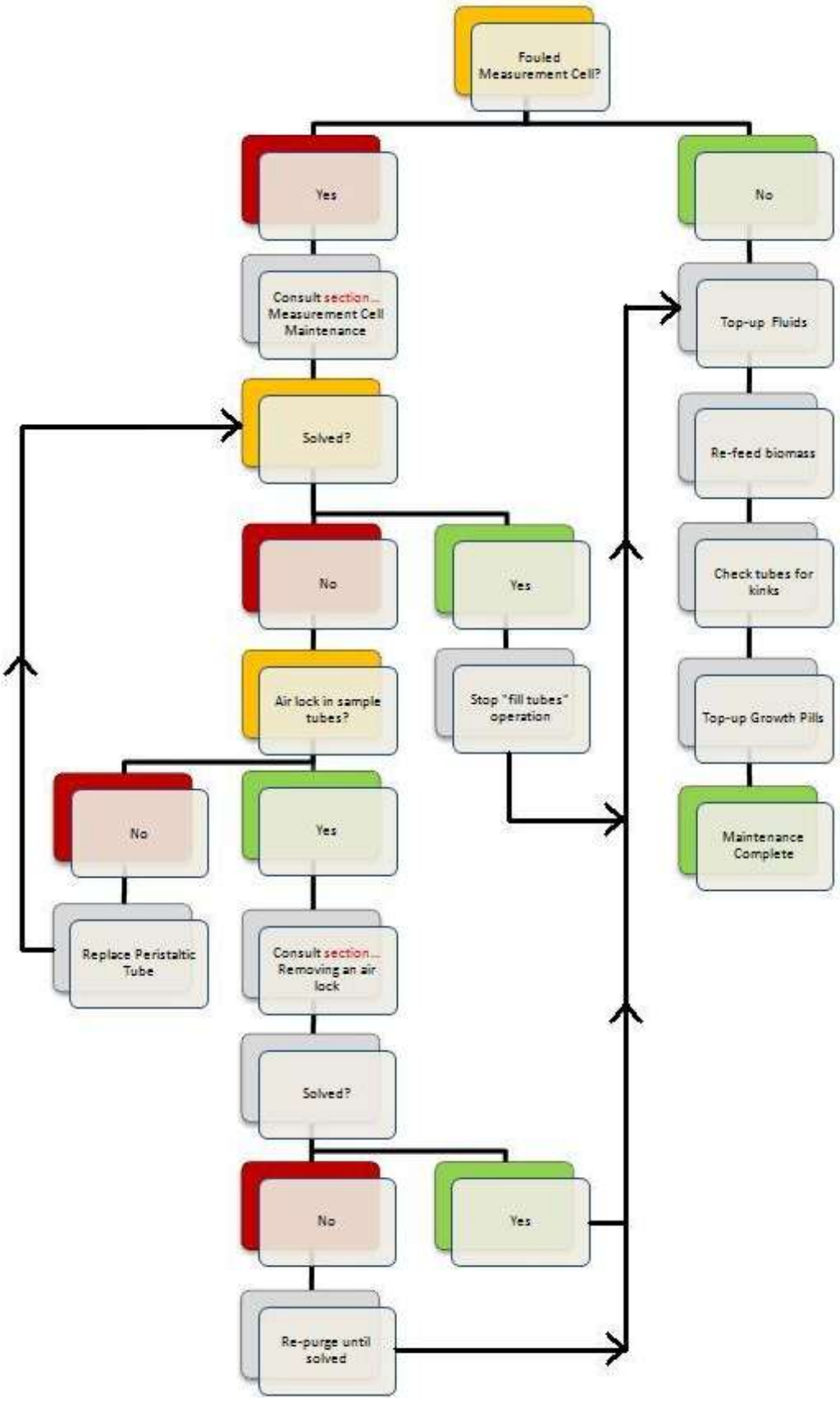
Weppen, P., Ebens, J., Müller, B.G. and Schuller, D. (1991), “On-line estimation of biological oxygen demand using direct calorimetry on surface attached microbial cultures”, *Thermochimica Acta*, Vol. 193, pp. 135–143.

Xiao, Y., De Araujo, C., Sze, C.C. and Stuckey, D.C. (2015), “Toxicity measurement in biological wastewater treatment processes: a review.”, *Journal of Hazardous Materials*, Vol. 286, pp. 15–29.

You, S.-J., Tsai, Y.-P. and Huang, R.-Y. (2009), “Effect of heavy metals on nitrification performance in different activated sludge processes.”, *Journal of Hazardous Materials*, Vol. 165 No. 1-3, pp. 987–94.

APPENDICES

Appendix 1 – Nitritox maintenance regime



Appendix 2 – Probe fouling in sewer environment

Appendix 2a Fouled DO probe from sewer placement.

Appendix 2b The sewer is a harsh environment for sensor placement with many solids types and sizes. Pictured is driftwood, rags, plastics, fats, oils and greases in a sewage pumping station wet well, Trent Vale, Stoke-on-Trent, UK. To the left is a small submersible pump completely enveloped in rags.

Appendix 3 – CFBBR calculations

Biomass production rate

$$\mathbf{m = YQ(s_i - s)} \qquad \mathbf{(4.12)}$$

where m = biomass production rate, $kg.hr^{-1}$

s_i = influent concentration of limiting substrate, $kg\text{-}NH_4\text{-}N.m^{-3}$

s = effluent concentration of limiting substrate, $kg\text{-}NH_4\text{-}N.m^{-3}$

Y = yield coefficient, $kg_{biomass.}\ kg_{substrate}^{-1}$

Q = Flowrate = 0.042 $m^3.hr^{-1}$

$$s \approx s_i$$

$$m = 0.5 \times 0.042(27.5\text{-}27.4)$$

$$= 0.0021\ kg.hr^{-1}$$

i.e. low to limit over growth of new bacteria

Gas hold-up

$$\boldsymbol{\varepsilon_g = (10 + 5.54\varepsilon C_s)U_g^{1.44}} \qquad \mathbf{(4.13)}$$

where ε_g = overall gas hold-up, dimensionless

C_s = apparent solids hold-up = 14 %v/v

U_g = superficial gas velocity = 0.02 $m.s^{-1}$

$$\varepsilon_g = [10 + 5.54(14.3)]0.02^{1.44}$$

$$= 0.319$$

Volumetric oxygen mass transfer coefficient

$$\boldsymbol{k_L a = (1.61 \times 10^3) \frac{\varepsilon_g}{1-\varepsilon_g}} \quad \textbf{(4.14)}$$

where $k_L a$ = volumetric oxygen mass transfer coefficient, hr^{-1}

ε_g = overall gas hold-up, dimensionless

$$k_L a = (1.61 \times 10^3) \frac{0.319}{1 - 0.319}$$

$$= 754.59 \text{ hr}^{-1}$$

Henrys Law

$$\boldsymbol{C^*_{DO} = \frac{p}{H}} \quad \textbf{(4.15)}$$

where C^*_{DO} = saturated DO concentration, $mg.L^{-1}$

p = atmospheric partial pressure of oxygen = 0.21 atm

H = Henry's constant for oxygen = $0.024 atm.l.mg^{-1}$

$$C^*_{DO} = \frac{0.21}{0.024}$$

$$= 8.75 \text{ mg.L}^{-1}$$

Oxygen mass transfer rate

$$\mathbf{n = k_L a\,(C^*_{DO} - C_{DO})} \quad \textbf{(4.16)}$$

where n = oxygen mass transfer rate, $kg.m^{-3}.hr^{-1}$

$k_L a$ = oxygen transfer coefficient = 65338.2 hr^{-1}

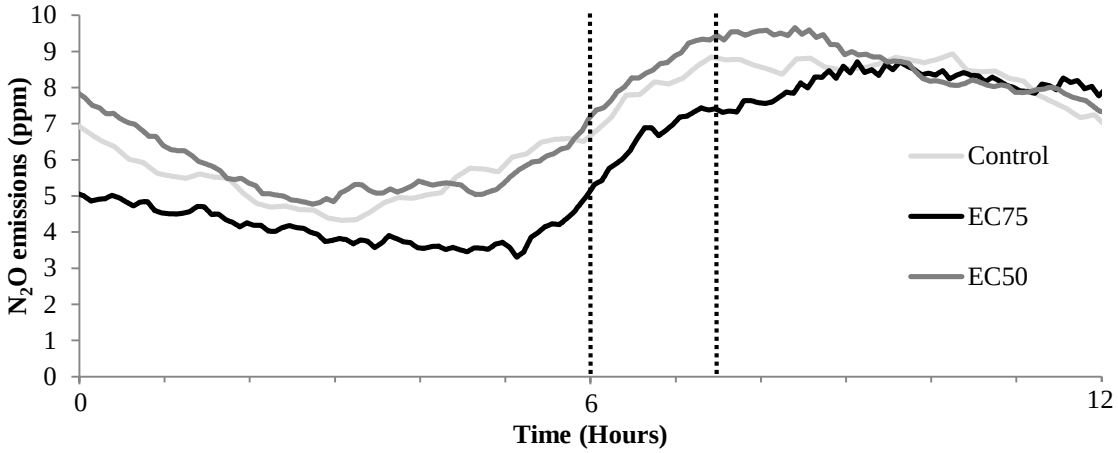
C_{DO} = DO concentration in effluent = 0.0025 $kg.m^{-3}$

C^*_{DO} = saturated DO concentration = 0.00875 $kg.m^{-3}$

$$n = 54.59\,(0.00875 - 0.0025)$$

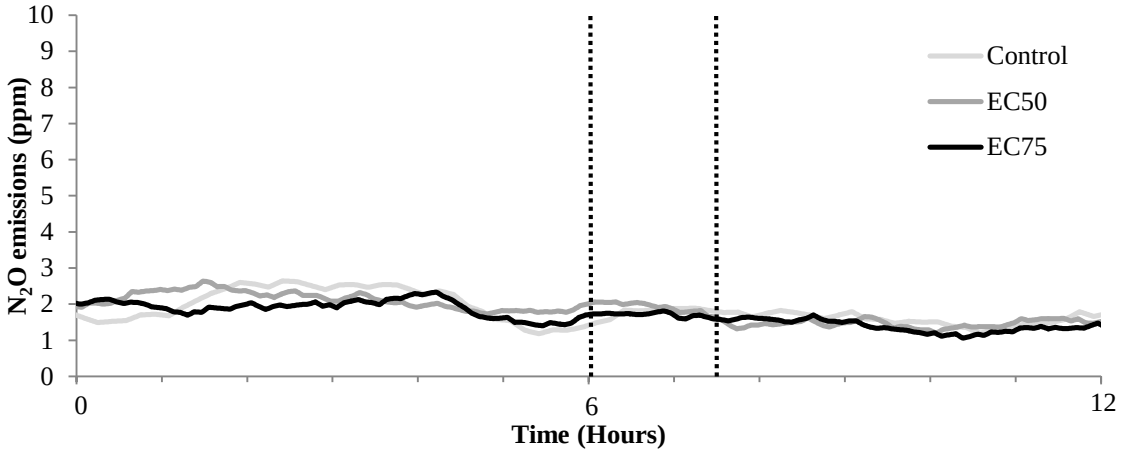
$$= 4.72 \text{ kg.m}^{-3}.\text{hr}^{-1}$$

Appendix 4 – Sewer biofilm response to chromium (VI)



Appendix 4 12 hour emissions profile for sewer biofilms conditioned with real sewage. A 90 minute 16 $mg.L^{-1}$ (EC_{50}) 64 $mg.L^{-1}$ (EC_{75}) chromium (VI) toxic shock was applied at hour 6 (within black dotted lines).

Appendix 5 – Sewer biofilm response to nickel (II)



Appendix 5 12 hour emissions profile for sewer biofilms conditioned with real sewage. A 90 minute 8 $mg.L^{-1}$ (EC_{50}) and 33 $mg.L^{-1}$ (EC_{75}) nickel (II) toxic shock was applied at hour 6 (within black dotted lines).

Appendix 6 – System A whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No.

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System A - 1 Inlet and 2 network CFBBR - Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£4.98	1.5	£39.75	£178.92
2	Data allowance	12	1GB per month data usage on Three network	£30.00	1.5	£39.75	£837.00
3	Check-up	12	General check-up and clean	£0.00	1.5	£39.75	£477.00
4	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£9.96	1.5	£39.75	£99.42
5	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Total Annual Cost **£1,745.34**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1	N-Tox		2 units at £18000ex VAT each	£36,000.00	1.0			
2	Vasaila CO2 monitor	10	2 units at £216ex VAT each	£432.00	1.0	£26.50	£458.50	10
3	Watson marlow 520R peristaltic pump	10	2 units at £1000ex VAT each	£2,000.00	1.0	£26.50	£2,026.50	10
4	Laptop	5	2 units at £300ex VAT each	£600.00	1.0	£26.50	£626.50	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	2 units at £30ex VAT each	£60.00	1.0	£26.50	£86.50	10
7	CFBBR Reactor		2 units at £127ex VAT each + 3hours to make	£413.00	6.0			
8	Pipes		2 units at £100ex VAT each	£200.00	3.0			
9	Supply tank		2 units at £50ex VAT each	£100.00	1.0			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	2 Units at £200ex Vat each from RS	£400.00	1.0	£26.50	£426.50	10
11	Fixings		2 units at £100ex VAT each	£200.00	1.0			
12	Clarke wiz air compressor pump for CFBBR aeration	10	2 units at £75ex Vat each	£150.00	1.0	£26.50	£176.50	10
13	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	2 Units at £71ex Vat each	£142.00	1.0	£26.50	£168.50	5 10 15
14	CO2 transmiter measurement cell		2 Units at £20ex VAT each + 1 hour to make	£40.00	2.0			
15	N-Tox gas sample pump	5	2 Units at £100ex VAT each	£200.00	1.0	£26.50	£226.50	5 10 15
16	Pico log data logger	10	2 Units at £159 each	£318.00	0.5	£13.25	£331.25	10
17								
18								
				£42,130.00	23.0	£238.50		
					£609.5			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	626.5	0	0	0	0	0	0	0	0	168.5	0	226.5	0	0	0	£1,021.50
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	458.5	2026.5	626.5	888.25	86.5	0	0	0	426.5	0	176.5	168.5	0	226.5	331.25	0	0	£5,415.50
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	626.5	0	0	0	0	0	0	0	0	168.5	0	226.5	0	0	0	£1,021.50
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		1	2	3
Duty Details		In-sewer EWS device - CFBBR reactor	Inlet works EWS device - CFBBR reactor	
kW consumed at Duty point		**0.5**	**2.0**	
Average operating hours per day at each duty	Mon	24.0	24.0	
	Tue	24.0	24.0	
	Wed	24.0	24.0	
	Thu	24.0	24.0	
	Fri	24.0	24.0	
	Sat	24.0	24.0	
	Sun	24.0	24.0	
Total operating hours per week		**168.0**	**168.0**	**0.0**
Total kWh per week		**83.7**	**335.7**	**0.0**
		Total kWh per Year		**21889**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- Inlet	Duty point 3 - N/A
N-Tox	2	0.15	0.15	0.15	
Vasaila CO2 monitor	2	0.0025	0.0025	0.0025	
520R peristaltic pump	2	0.135	0.135	0.135	
Laptop	2	0.065	0.065	0.065	
Submersible pump	1	1.5		1.5	
Autosampler pump	2	0.017	0.017	0.017	
CFBBR aeration	2	0.099	0.099	0.099	
CO2 sample pump	2	0.03	0.03	0.03	
		Total power consumption (kW)	0.4985	1.9985	0

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM A DETAILS

Scheme Name	In-sewer toxicity early warning system
STW Project No.	
Quotation / Spec / Proposal No.	

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		**20**	***Note***				
Interest Rate (i%)		**0.50**	***Note***				
Inflation Rate (p%)		**1.50**	***Note***				
Real Discount Rate (i-p)		**-1.0**					
Initial Investment Costs - Civil Works	*Cost 1*	**£0.00**	***Note***				
Initial Investment Costs - M&E Equipment	*Cost 2*	**£42,130.00**	***Note***				
M&E Installation and commissioning cost	*Cost 3*	**£14,043.33**					
Energy price (pence per kWh)		**8.0**	***Note***				
Total energy consumption per year (kWh)		**21889**	***Note***				
Energy cost per year	*Cost 4*	**£1,741.27**	***Note***				
Planned maintenance cost (cost per annum)	*Cost 5*	**£1,214.56**	***Note***				
Sum of yearly costs (4+5)	*Cost6*	**£2,955.83**	***Note***				
Present Value (PV) of yearly costs	*Cost 7*	**£65,806.52**	***Note***			**df**	**22.26**

YEAR		***Note***		***Note***		
Overhaul Costs; Repair, Replacement, etc						
2	*Cost 8*		*Cost 26*		**Cp/Cn**	**1.02**
3	*Cost 9*		*Cost 27*		**Cp/Cn**	**1.03**
4	*Cost 10*		*Cost 28*		**Cp/Cn**	**1.04**
5	*Cost 11*	**£1,021.50**	*Cost 29*	**£1,074.14**	**Cp/Cn**	**1.05**
6	*Cost 12*		*Cost 30*		**Cp/Cn**	**1.06**
7	*Cost 13*		*Cost 31*		**Cp/Cn**	**1.07**
8	*Cost 14*		*Cost 32*		**Cp/Cn**	**1.08**
9	*Cost 15*		*Cost 33*		**Cp/Cn**	**1.09**
10	*Cost 16*	**£5,415.50**	*Cost 34*	**£5,988.07**	**Cp/Cn**	**1.11**
11	*Cost 17*		*Cost 35*		**Cp/Cn**	**1.12**
12	*Cost 18*		*Cost 36*		**Cp/Cn**	**1.13**
13	*Cost 19*		*Cost 37*		**Cp/Cn**	**1.14**
14	*Cost 20*		*Cost 38*		**Cp/Cn**	**1.15**
15	*Cost 21*	**£1,021.50**	*Cost 39*	**£1,187.71**	**Cp/Cn**	**1.16**
16	*Cost 22*		*Cost 40*		**Cp/Cn**	**1.17**
17	*Cost 23*		*Cost 41*		**Cp/Cn**	**1.19**
18	*Cost 24*		*Cost 42*		**Cp/Cn**	**1.20**
19	*Cost 25*		*Cost 43*		**Cp/Cn**	**1.21**

Sum of Overhaul Costs			*Cost 44*	**£8,249.92**	
Present Value Life Cycle Cost (LCC) …		**£130,229.78**			
…of which:					
1 - Initial Investment Cost		**£56,173.33**	***Note***		
2 - Energy Cost		**£38,766.41**	***Note***		
3 - Planned Maintenance Cost		**£27,040.11**	***Note***		
4 - Overhaul Cost		**£8,249.92**	***Note***		

Appendix 7 – System B whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No.

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System B - 1 Inlet and 2 network CFBBR - Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£4.98	1.5	£39.75	£178.92
2	Data allowance	12	1GB per month data usage on Three network	£30.00	1.5	£39.75	£837.00
3	Check-up	12	General check-up and clean	£0.00	1.5	£39.75	£477.00
4	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£9.96	1.5	£39.75	£99.42
5	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							

Total Annual Cost **£1,745.34**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1	N-Tox		3 units at £18000ex VAT each	£54,000.00	1.5			
2	Vasaila CO2 monitor	10	3 units at £216ex VAT each	£648.00	1.5	£39.75	£687.75	10
3	Watson marlow 520R peristaltic pump	10	3 units at £1000ex VAT each	£3,000.00	1.5	£39.75	£3,039.75	10
4	Laptop	5	3 units at £300ex VAT each	£900.00	1.5	£39.75	£939.75	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	3 units at £30ex VAT each	£90.00	1.5	£39.75	£129.75	10
7	CFBBR Reactor		3 units at £127ex VAT each + 3 hrs to make	£619.50	9.0			
8	Pipes		3 units at £100ex VAT each	£300.00	4.5			
9	Supply tank		3 units at £50ex VAT each	£150.00	1.5			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	3 Units at £200ex Vat each from RS	£600.00	1.5	£39.75	£639.75	10
11	Fixings		3 units at £100ex VAT each	£300.00	1.5			
12	Clarke wiz air compressor pump for CFBBR aeration	10	3 units at £75ex Vat each	£225.00	1.5	£39.75	£264.75	10
13	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	3 Units at £71ex Vat each	£213.00	1.5	£39.75	£252.75	5 10 15
14	CO2 transmiter measurement cell		3 Units at £20ex VAT each + 1 hour to make	£60.00	3.0			
15	N-Tox gas sample pump	5	3 Units at £100ex VAT each	£300.00	1.5	£39.75	£339.75	5 10 15
16	Pico log data logger	10	3 Units at £159 each	£477.00	0.5	£13.25	£490.25	10
17								
18								
				£62,757.50	34.0	£344.50		
					901			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	939.75	0	0	0	0	0	0	0	0	252.75	0	339.75	0	0	0	£1,532.25
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	687.75	3039.8	939.75	888.25	129.75	0	0	0	639.75	0	264.75	252.75	0	339.75	490.25	0	0	£7,672.50
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	939.75	0	0	0	0	0	0	0	0	252.75	0	339.75	0	0	0	£1,532.25
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		**1**	**2**	**3**
Duty Details		In-sewer EWS device - CFBBR reactor	In-sewer EWS device - CFBBR reactor	Inlet works EWS device - CFBBR reactor
kW consumed at Duty point		**0.5**	**0.5**	**2.0**
Average operating hours per day at each duty	Mon	24.0	24.0	24.0
	Tue	24.0	24.0	24.0
	Wed	24.0	24.0	24.0
	Thu	24.0	24.0	24.0
	Fri	24.0	24.0	24.0
	Sat	24.0	24.0	24.0
	Sun	24.0	24.0	24.0
Total operating hours per week		**168.0**	**168.0**	**168.0**
Total kWh per week		**83.7**	**83.7**	**335.7**
		Total kWh per Year		**26259**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- In-sewer	Duty point 3 power consumption- Inlet
N-Tox	2	0.15	0.15	0.15	0.15
Vasaila CO2 monitor	2	0.0025	0.0025	0.0025	0.0025
520R peristaltic pump	2	0.135	0.135	0.135	0.135
Laptop	2	0.065	0.065	0.065	0.065
Submersible pump	1	1.5			1.5
Autosampler pump	2	0.017	0.017	0.017	0.017
CFBBR aeration	2	0.099	0.099	0.099	0.099
CO2 sample pump	2	0.03	0.03	0.03	0.03
		Total power consumption (kW)	0.4985	0.4985	1.9985

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM B DETAILS

Scheme Name	In-sewer toxicity early warning system						
STW Project No.				**Quotation / Spec / Proposal No.**	0		

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		20	*Note*
Interest Rate (i%)		0.50	*Note*
Inflation Rate (p%)		1.50	*Note*
Real Discount Rate (i-p)		-1.0	
Initial Investment Costs - Civil Works	*Cost 1*	£0.00	*Note*
Initial Investment Costs - M&E Equipment	*Cost 2*	£62,757.50	*Note*
M&E Installation and commissioning cost	*Cost 3*	£20,919.17	
Energy price (pence per kWh)		8.0	*Note*
Total energy consumption per year (kWh)		26259	*Note*
Energy cost per year	*Cost 4*	£2,088.90	*Note*
Planned maintenance cost (cost per annum)	*Cost 5*	£1,745.34	*Note*
Sum of yearly costs (4+5)	*Cost6*	£3,834.24	*Note*
Present Value (PV) of yearly costs	*Cost 7*	£85,362.91	*Note*

df 22.26

YEAR		*Note*		*Note*		
Overhaul Costs; Repair, Replacement, etc						
2	*Cost 8*		*Cost 26*		**Cp/Cn**	1.02
3	*Cost 9*		*Cost 27*		**Cp/Cn**	1.03
4	*Cost 10*		*Cost 28*		**Cp/Cn**	1.04
5	*Cost 11*	£1,532.25	*Cost 29*	£1,611.22	**Cp/Cn**	1.05
6	*Cost 12*		*Cost 30*		**Cp/Cn**	1.06
7	*Cost 13*		*Cost 31*		**Cp/Cn**	1.07
8	*Cost 14*		*Cost 32*		**Cp/Cn**	1.08
9	*Cost 15*		*Cost 33*		**Cp/Cn**	1.09
10	*Cost 16*	£7,672.50	*Cost 34*	£8,483.69	**Cp/Cn**	1.11
11	*Cost 17*		*Cost 35*		**Cp/Cn**	1.12
12	*Cost 18*		*Cost 36*		**Cp/Cn**	1.13
13	*Cost 19*		*Cost 37*		**Cp/Cn**	1.14
14	*Cost 20*		*Cost 38*		**Cp/Cn**	1.15
15	*Cost 21*	£1,532.25	*Cost 39*	£1,781.57	**Cp/Cn**	1.16
16	*Cost 22*		*Cost 40*		**Cp/Cn**	1.17
17	*Cost 23*		*Cost 41*		**Cp/Cn**	1.19
18	*Cost 24*		*Cost 42*		**Cp/Cn**	1.20
19	*Cost 25*		*Cost 43*		**Cp/Cn**	1.21

Sum of Overhaul Costs			*Cost 44*	£11,876.47
Present Value Life Cycle Cost (LCC) …		£180,916.05		
…of which:				
1 - Initial Investment Cost		£83,676.67	*Note*	
2 - Energy Cost		£46,505.88	*Note*	
3 - Planned Maintenance Cost		£38,857.03	*Note*	
4 - Overhaul Cost		£11,876.47	*Note*	

Appendix 8 – System C whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No. 0

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System C - Inlet and 1 network CFBBR- Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£1.66	0.5	£13.25	£59.64
2	Data allowance	12	1GB per month data usage on Three network	£20.00	1.0	£26.50	£558.00
3	CFBBR Check-up	12	General check-up and clean	£0.00	0.5	£13.25	£159.00
4	Nitritox maintenance	52	Refill fluids and general check-up / clean	£20.00	1.0	£26.50	£2,418.00
5	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£3.32	0.5	£13.25	£33.14
6	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
7							
8							
9							
10							
11							
12							
13							
14							
15							
						Total Annual Cost	**£3,380.78**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1	N-Tox		1 unit at £18000ex VAT each	£18,000.00	0.5			
2	Vasaila CO2 monitor	10	1 unit at £216ex VAT each	£216.00	0.5	£13.25	£229.25	10
3	Watson marlow 520R peristaltic pump	10	1 unit at £1000ex VAT each	£1,000.00	0.5	£13.25	£1,013.25	10
4	Laptop	5	2 units at £300ex VAT each	£600.00	1.0	£26.50	£626.50	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	2 units at £30ex VAT each	£60.00	1.0	£26.50	£86.50	10
7	CFBBR Reactor		1 unit at £127ex VAT each + 3 hrs to make	£206.50	3.0			
8	Pipes		2 units at £100ex VAT each	£200.00	4.5			
9	Supply tank		2 units at £50ex VAT each	£100.00	1.0			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	2 Units at £200ex Vat each from RS	£400.00	1.0	£26.50	£426.50	10
11	Fixings		2 units at £100ex VAT each	£200.00	1.0			
12	Nitritox inlet works monitor		1 unit at £22000ex VAT each	£22,000.00	2.0			
13	Clarke wiz air compressor pump for CFBBR aeration	10	1 unit at £75ex Vat each	£75.00	0.5	£13.25	£88.25	10
14	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	1 Unit at £71ex Vat each	£71.00	0.5	£13.25	£84.25	5 10 15
15	CO2 transmiter measurement cell		1 Unit at £20ex VAT each + 1 hour to make	£20.00	1.0			
16	N-Tox gas sample pump	5	1 Unit at £100ex VAT each	£100.00	0.5	£13.25	£113.25	5 10 15
17	Pico log data logger	10	2 Units at £159 each	£318.00	0.5	£13.25	£331.25	10
18								
				£44,441.50	19.5	£172.25		
					516.75			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	626.5	0	0	0	0	0	0	0	0	0	84.25	0	113.25	0	0	£824.00
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	229.25	1013.3	626.5	888.25	86.5	0	0	0	426.5	0	0	88.25	84.25	0	113.25	331.25	0	£3,887.25
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	626.5	0	0	0	0	0	0	0	0	0	84.25	0	113.25	0	0	£824.00
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		1	2	3
Duty Details		In-sewer EWS device - CFBBR reactor	Inlet works EWS device - Nitritox	
kW consumed at Duty point		**0.5**	**1.7**	
Average operating hours per day at each duty	Mon	24.0	24.0	
	Tue	24.0	24.0	
	Wed	24.0	24.0	
	Thu	24.0	24.0	
	Fri	24.0	24.0	
	Sat	24.0	24.0	
	Sun	24.0	24.0	
Total operating hours per week		**168.0**	**168.0**	**0.0**
Total kWh per week		**83.7**	**282.6**	**0.0**
		Total kWh per Year		**19115**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- Inlet	Duty point 3 - N/A
N-Tox	2	0.15	0.15		
Vasaila CO2 monitor	2	0.0025	0.0025		
520R peristaltic pump	2	0.135	0.135		
Laptop	2	0.065	0.065	0.065	
Submersible pump	1	1.5		1.5	
Autosampler pump	2	0.017	0.017	0.017	
Nitritox	1	0.1		0.1	
CFBBR aeration	2	0.099	0.099		
CO2 sample pump	2	0.03	0.03		
		Total power consumption (kW)	0.4985	1.682	0

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM C DETAILS

Scheme Name	In-sewer toxicity early warning system
STW Project No.	0
Quotation / Spec / Proposal No.	0

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		**20**	***Note***	
Interest Rate (i%)		**0.50**	***Note***	Sep-14
Inflation Rate (p%)		**1.50**	***Note***	Sep-14
Real Discount Rate (i-p)		**-1.0**		
Initial Investment Costs - Civil Works	*Cost 1*	**£0.00**	***Note***	
Initial Investment Costs - M&E Equipment	*Cost 2*	**£44,441.50**	***Note***	
M&E Installation and commissioning cost	*Cost 3*	**£14,813.83**		
Energy price (pence per kWh)		**8.0**	***Note***	
Total energy consumption per year (kWh)		**19115**	***Note***	
Energy cost per year	*Cost 4*	**£1,520.60**	***Note***	
Planned maintenance cost (cost per annum)	*Cost 5*	**£3,380.78**	***Note***	
Sum of yearly costs (4+5)	*Cost6*	**£4,901.38**	***Note***	
Present Value (PV) of yearly costs	*Cost 7*	**£109,120.85**	***Note***	**df** **22.26**

YEAR		***Note***		***Note***		
Overhaul Costs; Repair, Replacement, etc						
2	*Cost 8*		*Cost 26*		**Cp/Cn**	**1.02**
3	*Cost 9*		*Cost 27*		**Cp/Cn**	**1.03**
4	*Cost 10*		*Cost 28*		**Cp/Cn**	**1.04**
5	*Cost 11*	**£824.00**	*Cost 29*	**£866.47**	**Cp/Cn**	**1.05**
6	*Cost 12*		*Cost 30*		**Cp/Cn**	**1.06**
7	*Cost 13*		*Cost 31*		**Cp/Cn**	**1.07**
8	*Cost 14*		*Cost 32*		**Cp/Cn**	**1.08**
9	*Cost 15*		*Cost 33*		**Cp/Cn**	**1.09**
10	*Cost 16*	**£3,887.25**	*Cost 34*	**£4,298.24**	**Cp/Cn**	**1.11**
11	*Cost 17*		*Cost 35*		**Cp/Cn**	**1.12**
12	*Cost 18*		*Cost 36*		**Cp/Cn**	**1.13**
13	*Cost 19*		*Cost 37*		**Cp/Cn**	**1.14**
14	*Cost 20*		*Cost 38*		**Cp/Cn**	**1.15**
15	*Cost 21*	**£824.00**	*Cost 39*	**£958.07**	**Cp/Cn**	**1.16**
16	*Cost 22*		*Cost 40*		**Cp/Cn**	**1.17**
17	*Cost 23*		*Cost 41*		**Cp/Cn**	**1.19**
18	*Cost 24*		*Cost 42*		**Cp/Cn**	**1.20**
19	*Cost 25*		*Cost 43*		**Cp/Cn**	**1.21**
Sum of Overhaul Costs			*Cost 44*	**£6,122.78**		

Present Value Life Cycle Cost (LCC) …	**£174,498.96**	
…of which:		
1 - Initial Investment Cost	**£59,255.33**	***Note***
2 - Energy Cost	**£33,853.53**	***Note***
3 - Planned Maintenance Cost	**£75,267.31**	***Note***
4 - Overhaul Cost	**£6,122.78**	***Note***

Appendix 9 – System D whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No.

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System D - 1 Inlet and 2 network CFBBR - Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	CFBBR- 0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£3.32	1.0	£26.50	£119.28
2	Data allowance	12	1GB per month data usage on Three network	£30.00	1.5	£39.75	£837.00
3	CFBBR Check-up	12	General check-up and clean	£0.00	1.0	£26.50	£318.00
4	Nitritox maintenance	52	Refill fluids and general check-up	£20.00	1.0	£26.50	£2,418.00
5	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£6.64	1.0	£26.50	£66.28
6	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
7							
8							
9							
10							
11							
12							
13							
14							
15							
						Total Annual Cost	**£3,911.56**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1	N-Tox		2 units at £18000ex VAT each	£36,000.00	1.0			
2	Vasaila CO2 monitor	10	2 units at £216ex VAT each	£432.00	1.0	£26.50	£458.50	10
3	Watson marlow 520R peristaltic pump	10	2 units at £1000ex VAT each	£2,000.00	1.0	£26.50	£2,026.50	10
4	Laptop	5	3 units at £300ex VAT each	£900.00	1.5	£39.75	£939.75	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	3 units at £30ex VAT each	£90.00	1.5	£39.75	£129.75	10
7	CFBBR Reactor		2 units at £127ex VAT each + 3 hrs to make	£413.00	6.0			
8	Pipes		3 units at £100ex VAT each	£300.00	4.5			
9	Supply tank		3 units at £50ex VAT each	£150.00	1.5			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	3 Units at £200ex Vat each from RS	£600.00	1.5	£39.75	£639.75	10
11	Fixings		3 units at £100ex VAT each	£300.00	1.5			
12	Nitritox inlet works monitor		1 unit at £22000ex VAT each	£22,000.00	2.0			
13	Clarke wiz air compressor pump for CFBBR aeration	10	2 units at £75ex Vat each	£150.00	1.0	£26.50	£176.50	10
14	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	2 Units at £71ex Vat each	£142.00	1.0	£26.50	£168.50	5 10 15
15	CO2 transmiter measurement cell		2 Units at £20ex VAT each + 1 hour to make	£40.00	2.0			
16	N-Tox gas sample pump	5	2 Units at £100ex VAT each	£200.00	1.0	£26.50	£226.50	5 10 15
17	Pico log data logger	10	3 Units at £159 each	£477.00	0.5	£13.25	£490.25	10
18								
				£65,069.00	29.0	£278.25		
					768.5			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	939.75	0	0	0	0	0	0	0	0	0	168.5	0	226.5	0	0	£1,334.75
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	458.5	2026.5	939.75	888.25	129.75	0	0	0	639.75	0	0	176.5	168.5	0	226.5	490.25	0	£6,144.25
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	939.75	0	0	0	0	0	0	0	0	0	168.5	0	226.5	0	0	£1,334.75
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		1	2	3
Duty Details		In-sewer EWS device - CFBBR reactor	In-sewer EWS device - CFBBR reactor	Inlet works EWS device - Nitritox
kW consumed at Duty point		**0.5**	**0.5**	**1.7**
Average operating hours per day at each duty	Mon	24.0	24.0	24.0
	Tue	24.0	24.0	24.0
	Wed	24.0	24.0	24.0
	Thu	24.0	24.0	24.0
	Fri	24.0	24.0	24.0
	Sat	24.0	24.0	24.0
	Sun	24.0	24.0	24.0
Total operating hours per week		**168.0**	**168.0**	**168.0**
Total kWh per week		**83.7**	**83.7**	**282.6**
		Total kWh per Year		**23485**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- In-sewer	Duty point 3 power consumption- Inlet
N-Tox	2	0.15	0.15	0.15	
Vasaila CO2 monitor	2	0.0025	0.0025	0.0025	
520R peristaltic pump	2	0.135	0.135	0.135	
Laptop	2	0.065	0.065	0.065	0.065
Submersible pump	1	1.5			1.5
Autosampler pump	2	0.017	0.017	0.017	0.017
Nitritox	1	0.1			0.1
CFBBR aeration	2	0.099	0.099	0.099	
CO2 sample pump	2	0.03	0.03	0.03	
		Total power consumption (kW)	0.4985	0.4985	1.682

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM D DETAILS

Scheme Name	In-sewer toxicity early warning system						
STW Project No.				**Quotation / Spec / Proposal No.**			

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		**20**	***Note***		
Interest Rate (i%)		**0.50**	***Note***	Sep-14	
Inflation Rate (p%)		**1.50**	***Note***	Sep-14	
Real Discount Rate (i-p)		**-1.0**			
Initial Investment Costs - Civil Works	*Cost 1*	**£0.00**	***Note***		
Initial Investment Costs - M&E Equipment	*Cost 2*	**£65,069.00**	***Note***		
M&E Installation and commissioning cost	*Cost 3*	**£21,689.67**			
Energy price (pence per kWh)		**8.0**	***Note***		
Total energy consumption per year (kWh)		**23485**	***Note***		
Energy cost per year	*Cost 4*	**£1,868.23**	***Note***		
Planned maintenance cost (cost per annum)	*Cost 5*	**£3,911.56**	***Note***		
Sum of yearly costs (4+5)	*Cost6*	**£5,779.79**	***Note***		
Present Value (PV) of yearly costs	*Cost 7*	**£128,677.23**	***Note***	**df**	**22.26**

	YEAR		***Note***		***Note***		
Overhaul Costs; Repair, Replacement, etc	**2**	*Cost 8*		*Cost 26*		**Cp/Cn**	**1.02**
	3	*Cost 9*		*Cost 27*		**Cp/Cn**	**1.03**
	4	*Cost 10*		*Cost 28*		**Cp/Cn**	**1.04**
	5	*Cost 11*	**£1,334.75**	*Cost 29*	**£1,403.54**	**Cp/Cn**	**1.05**
	6	*Cost 12*		*Cost 30*		**Cp/Cn**	**1.06**
	7	*Cost 13*		*Cost 31*		**Cp/Cn**	**1.07**
	8	*Cost 14*		*Cost 32*		**Cp/Cn**	**1.08**
	9	*Cost 15*		*Cost 33*		**Cp/Cn**	**1.09**
	10	*Cost 16*	**£6,144.25**	*Cost 34*	**£6,793.87**	**Cp/Cn**	**1.11**
	11	*Cost 17*		*Cost 35*		**Cp/Cn**	**1.12**
	12	*Cost 18*		*Cost 36*		**Cp/Cn**	**1.13**
	13	*Cost 19*		*Cost 37*		**Cp/Cn**	**1.14**
	14	*Cost 20*		*Cost 38*		**Cp/Cn**	**1.15**
	15	*Cost 21*	**£1,334.75**	*Cost 39*	**£1,551.93**	**Cp/Cn**	**1.16**
	16	*Cost 22*		*Cost 40*		**Cp/Cn**	**1.17**
	17	*Cost 23*		*Cost 41*		**Cp/Cn**	**1.19**
	18	*Cost 24*		*Cost 42*		**Cp/Cn**	**1.20**
	19	*Cost 25*		*Cost 43*		**Cp/Cn**	**1.21**

Sum of Overhaul Costs			*Cost 44*	**£9,749.33**	

Present Value Life Cycle Cost (LCC) …	**£225,185.23**		
…of which:			
1 - Initial Investment Cost	**£86,758.67**	***Note***	
2 - Energy Cost	**£41,593.00**	***Note***	
3 - Planned Maintenance Cost	**£87,084.23**	***Note***	
4 - Overhaul Cost	**£9,749.33**	***Note***	

Appendix 10 – System E whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No. 0

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System E- Inlet and 1 network CFBBR- Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£1.66	0.5	£13.25	£59.64
2	Data allowance	12	1GB per month data usage on Three network	£20.00	1.0	£26.50	£558.00
3	CFBBR Check-up	12	General check-up and clean	£0.00	0.5	£13.25	£159.00
4	Nitritox maintenance	52	Refill fluids and general check-up / clean	£20.00	1.0	£26.50	£2,418.00
5	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£3.32	0.5	£13.25	£33.14
6	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
7							
8							
9							
10							
11							
12							
13							
14							
15							
						Total Annual Cost	**£3,380.78**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1								
2	Vasaila CO2 monitor	10	1 unit at £216ex VAT each	£216.00	0.5	£13.25	£229.25	10
3	Watson marlow 520R peristaltic pump	10	1 unit at £1000ex VAT each	£1,000.00	0.5	£13.25	£1,013.25	10
4	Laptop	5	2 units at £300ex VAT each	£600.00	1.0	£26.50	£626.50	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	2 units at £30ex VAT each	£60.00	1.0	£26.50	£86.50	10
7	CFBBR Reactor		1 unit at £127ex VAT each + 3 hrs to make	£206.50	3.0			
8	Pipes		2 units at £100ex VAT each	£200.00	4.5			
9	Supply tank		2 units at £50ex VAT each	£100.00	1.0			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	2 Units at £200ex Vat each from RS	£400.00	1.0	£26.50	£426.50	10
11	Fixings		2 units at £100ex VAT each	£200.00	1.0			
12	Nitritox inlet works monitor		1 unit at £22000ex VAT each	£22,000.00	2.0			
13	Clarke wiz air compressor pump for CFBBR aeration	10	1 unit at £75ex Vat each	£75.00	0.5	£13.25	£88.25	10
14	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	1 Unit at £71ex Vat each	£71.00	0.5	£13.25	£84.25	5 10 15
15	CO2 transmiter measurement cell		1 Unit at £20ex VAT each + 1 hour to make	£20.00	1.0			
16								
17	Pico log data logger	10	2 Units at £159 each	£318.00	0.5	£13.25	£331.25	10
18								
				£26,341.50	18.5	£159.00		
					490.25			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	626.5	0	0	0	0	0	0	0	0	0	84.25	0	0	0	0	£710.75
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	229.25	1013.3	626.5	888.25	86.5	0	0	0	426.5	0	0	88.25	84.25	0	0	331.25	0	£3,774.00
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	626.5	0	0	0	0	0	0	0	0	0	84.25	0	0	0	0	£710.75
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		1	2	3
Duty Details		In-sewer EWS device - CFBBR reactor	Inlet works EWS device - Nitritox	
kW consumed at Duty point		**0.3**	**1.7**	
Average operating hours per day at each duty	Mon	24.0	24.0	
	Tue	24.0	24.0	
	Wed	24.0	24.0	
	Thu	24.0	24.0	
	Fri	24.0	24.0	
	Sat	24.0	24.0	
	Sun	24.0	24.0	
Total operating hours per week		**168.0**	**168.0**	**0.0**
Total kWh per week		**58.5**	**282.6**	**0.0**
		Total kWh per Year		**17800**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- Inlet	Duty point 3 - N/A
Vasaila CO2 monitor	2	0.0025	0.0025		
520R peristaltic pump	2	0.135	0.135		
Laptop	2	0.065	0.065	0.065	
Submersible pump	1	1.5		1.5	
Autosampler pump	2	0.017	0.017	0.017	
Nitritox	1	0.1		0.1	
CFBBR aeration	2	0.099	0.099		
CO2 sample pump	2	0.03	0.03		
		Total power consumption (kW)	0.3485	1.682	0

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM E DETAILS

Scheme Name	In-sewer toxicity early warning system
STW Project No.	0
Quotation / Spec / Proposal No.	0

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		**20**	***Note***	
Interest Rate (i%)		**0.50**	***Note***	Sep-14
Inflation Rate (p%)		**1.50**	***Note***	Sep-14
Real Discount Rate (i-p)		**-1.0**		
Initial Investment Costs - Civil Works	*Cost 1*	**£0.00**	***Note***	
Initial Investment Costs - M&E Equipment	*Cost 2*	**£26,341.50**	***Note***	
M&E Installation and commissioning cost	*Cost 3*	**£8,780.50**		
Energy price (pence per kWh)		**8.0**	***Note***	
Total energy consumption per year (kWh)		**17800**	***Note***	
Energy cost per year	*Cost 4*	**£1,415.99**	***Note***	
Planned maintenance cost (cost per annum)	*Cost 5*	**£3,380.78**	***Note***	
Sum of yearly costs (4+5)	*Cost6*	**£4,796.77**	***Note***	
Present Value (PV) of yearly costs	*Cost 7*	**£106,791.92**	***Note***	**df** **22.26**

YEAR		***Note***		***Note***		
2	*Cost 8*		*Cost 26*		**Cp/Cn**	**1.02**
3	*Cost 9*		*Cost 27*		**Cp/Cn**	**1.03**
4	*Cost 10*		*Cost 28*		**Cp/Cn**	**1.04**
5	*Cost 11*	**£710.75**	*Cost 29*	**£747.38**	**Cp/Cn**	**1.05**
6	*Cost 12*		*Cost 30*		**Cp/Cn**	**1.06**
7	*Cost 13*		*Cost 31*		**Cp/Cn**	**1.07**
8	*Cost 14*		*Cost 32*		**Cp/Cn**	**1.08**
9	*Cost 15*		*Cost 33*		**Cp/Cn**	**1.09**
10	*Cost 16*	**£3,774.00**	*Cost 34*	**£4,173.02**	**Cp/Cn**	**1.11**
11	*Cost 17*		*Cost 35*		**Cp/Cn**	**1.12**
12	*Cost 18*		*Cost 36*		**Cp/Cn**	**1.13**
13	*Cost 19*		*Cost 37*		**Cp/Cn**	**1.14**
14	*Cost 20*		*Cost 38*		**Cp/Cn**	**1.15**
15	*Cost 21*	**£710.75**	*Cost 39*	**£826.40**	**Cp/Cn**	**1.16**
16	*Cost 22*		*Cost 40*		**Cp/Cn**	**1.17**
17	*Cost 23*		*Cost 41*		**Cp/Cn**	**1.19**
18	*Cost 24*		*Cost 42*		**Cp/Cn**	**1.20**
19	*Cost 25*		*Cost 43*		**Cp/Cn**	**1.21**

Overhaul Costs; Repair, Replacement, etc

Sum of Overhaul Costs *Cost 44* **£5,746.79**

Present Value Life Cycle Cost (LCC) …	**£147,660.71**	
…of which:		
1 - Initial Investment Cost	**£35,122.00**	***Note***
2 - Energy Cost	**£31,524.61**	***Note***
3 - Planned Maintenance Cost	**£75,267.31**	***Note***
4 - Overhaul Cost	**£5,746.79**	***Note***

Appendix 11 – System F whole life cost analysis

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS:

Scheme Name In-sewer toxicity early warning system

STW Project No.

Quotation / Spec / Proposal No.

Total Life Cycle Costs - System F - 1 Inlet and 2 network CFBBR - Maintenance Costs Build-up

STW Maintenance Labour Rate per hour £26.50 ***Note***

Asset Life 20

Planned Maintenance Events (Cost per Annum)

Task	Description of Work	Frequency per Annum	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Total Cost
1	CFBBR- 0.3m of Peristaltic tubing	4	£83ex Vat cost for 15m of 9.5mm ID from RS	£3.32	1.0	£26.50	£119.28
2	Data allowance	12	1GB per month data usage on Three network	£30.00	1.5	£39.75	£837.00
3	CFBBR Check-up	12	General check-up and clean	£0.00	1.0	£26.50	£318.00
4	Nitritox maintenance	52	Refill fluids and general check-up	£20.00	1.0	£26.50	£2,418.00
5	2 litres Kalnes media per CFBBR, £1.66ex VAT each	2	Required for re-seeds following toxic events	£6.64	1.0	£26.50	£66.28
6	Submersible pump service	2		£50.00	1.0	£26.50	£153.00
7							
8							
9							
10							
11							
12							
13							
14							
15							
						Total Annual Cost	**£3,911.56**

Overhaul Events

Task	Description of Work	Frequency in Years	Description of Spare Parts Required	Spares Cost per Task	Labour Hours per Task	Labour Cost	Cost per Task	Year in which task is performed
1								
2	Vasaila CO2 monitor	10	2 units at £216ex VAT each	£432.00	1.0	£26.50	£458.50	10
3	Watson marlow 520R peristaltic pump	10	2 units at £1000ex VAT each	£2,000.00	1.0	£26.50	£2,026.50	10
4	Laptop	5	3 units at £300ex VAT each	£900.00	1.5	£39.75	£939.75	5 10 15
5	DGO200/2/80 1pH 230V 50Hz submersible pump (inlet works only)	10	1 unit at £875ex VAT each from GM Treble	£875.00	0.5	£13.25	£888.25	10
6	Internet dongle	10	3 units at £30ex VAT each	£90.00	1.5	£39.75	£129.75	10
7	CFBBR Reactor		2 units at £127ex VAT each + 3 hrs to make	£413.00	6.0			
8	Pipes		3 units at £100ex VAT each	£300.00	4.5			
9	Supply tank		3 units at £50ex VAT each	£150.00	1.5			
10	Vandeflex AU EZ peristaltic pump for autostampler setup	10	3 Units at £200ex Vat each from RS	£600.00	1.5	£39.75	£639.75	10
11	Fixings		3 units at £100ex VAT each	£300.00	1.5			
12	Nititox inlet works monitor		1 unit at £22000ex VAT each	£22,000.00	2.0			
13	Clarke wiz air compressor pump for CFBBR aeration	10	2 units at £75ex Vat each	£150.00	1.0	£26.50	£176.50	10
14	Xylem FLOJET RLF12220D gas sample pump for CO2 transmitter	5	2 Units at £71ex Vat each	£142.00	1.0	£26.50	£168.50	5 10 15
15	CO2 transmiter measurement cell		2 Units at £20ex VAT each + 1 hour to make	£40.00	2.0			
16								
17	Pico log data logger	10	3 Units at £159 each	£477.00	0.5	£13.25	£490.25	10
18								
				£28,869.00	27.0	£251.75		
					715.5			

Overhaul Events (cont.)

Year of Event	Cost per task T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13	T14	T15	T16	T17	T18	Total Cost for the Year
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
5	0	0	0	939.75	0	0	0	0	0	0	0	0	0	168.5	0	0	0	0	£1,108.25
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	458.5	2026.5	939.75	888.25	129.75	0	0	0	639.75	0	0	176.5	168.5	0	0	490.25	0	£5,917.75
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
15	0	0	0	939.75	0	0	0	0	0	0	0	0	0	168.5	0	0	0	0	£1,108.25
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

Average Energy Consumption Calculator

Duty Number		1	2	3
Duty Details		In-sewer EWS device - CFBBR reactor	In-sewer EWS device - CFBBR reactor	Inlet works EWS device - Nitritox
kW consumed at Duty point		**0.3**	**0.3**	**1.7**
Average operating hours per day at each duty	Mon	24.0	24.0	24.0
	Tue	24.0	24.0	24.0
	Wed	24.0	24.0	24.0
	Thu	24.0	24.0	24.0
	Fri	24.0	24.0	24.0
	Sat	24.0	24.0	24.0
	Sun	24.0	24.0	24.0
Total operating hours per week		**168.0**	**168.0**	**168.0**
Total kWh per week		**58.5**	**58.5**	**282.6**
		Total kWh per Year		**20855**

Device	**Number of Units**	**Power consumption (kW) per unit**	Duty point 1 power consumption- In-sewer	Duty point 2 power consumption- In-sewer	Duty point 3 power consumption- Inlet
Vasaila CO2 monitor	2	0.0025	0.0025	0.0025	
520R peristaltic pump	2	0.135	0.135	0.135	
Laptop	2	0.065	0.065	0.065	0.065
Submersible pump	1	1.5			1.5
Autosampler pump	2	0.017	0.017	0.017	0.017
Nitritox	1	0.1			0.1
CFBBR aeration	2	0.099	0.099	0.099	
CO2 sample pump	2	0.03	0.03	0.03	
		Total power consumption (kW)	0.3485	0.3485	1.682

SEVERN TRENT WATER - LIFE CYCLE COSTING ANALYSIS: SYSTEM F DETAILS

Scheme Name	In-sewer toxicity early warning system						
STW Project No.				**Quotation / Spec / Proposal No.**			

Total Life Cycle Costs

Place cursor here for background information!

Asset Life (n)		**20**	***Note***		
Interest Rate (i%)		**0.50**	***Note***	Sep-14	
Inflation Rate (p%)		**1.50**	***Note***	Sep-14	
Real Discount Rate (i-p)		**-1.0**			
Initial Investment Costs - Civil Works	*Cost 1*	**£0.00**	***Note***		
Initial Investment Costs - M&E Equipment	*Cost 2*	**£28,869.00**	***Note***		
M&E Installation and commissioning cost	*Cost 3*	**£9,623.00**			
Energy price (pence per kWh)		**8.0**	***Note***		
Total energy consumption per year (kWh)		**20855**	***Note***		
Energy cost per year	*Cost 4*	**£1,659.02**	***Note***		
Planned maintenance cost (cost per annum)	*Cost 5*	**£3,911.56**	***Note***		
Sum of yearly costs (4+5)	*Cost6*	**£5,570.58**	***Note***		
Present Value (PV) of yearly costs	*Cost 7*	**£124,019.38**	***Note***	**df**	**22.26**

	YEAR		***Note***		***Note***		
Overhaul Costs;	**2**	*Cost 8*		*Cost 26*		**Cp/Cn**	**1.02**
Repair, Replacement, etc	**3**	*Cost 9*		*Cost 27*		**Cp/Cn**	**1.03**
	4	*Cost 10*		*Cost 28*		**Cp/Cn**	**1.04**
	5	*Cost 11*	**£1,108.25**	*Cost 29*	**£1,165.36**	**Cp/Cn**	**1.05**
	6	*Cost 12*		*Cost 30*		**Cp/Cn**	**1.06**
	7	*Cost 13*		*Cost 31*		**Cp/Cn**	**1.07**
	8	*Cost 14*		*Cost 32*		**Cp/Cn**	**1.08**
	9	*Cost 15*		*Cost 33*		**Cp/Cn**	**1.09**
	10	*Cost 16*	**£5,917.75**	*Cost 34*	**£6,543.42**	**Cp/Cn**	**1.11**
	11	*Cost 17*		*Cost 35*		**Cp/Cn**	**1.12**
	12	*Cost 18*		*Cost 36*		**Cp/Cn**	**1.13**
	13	*Cost 19*		*Cost 37*		**Cp/Cn**	**1.14**
	14	*Cost 20*		*Cost 38*		**Cp/Cn**	**1.15**
	15	*Cost 21*	**£1,108.25**	*Cost 39*	**£1,288.58**	**Cp/Cn**	**1.16**
	16	*Cost 22*		*Cost 40*		**Cp/Cn**	**1.17**
	17	*Cost 23*		*Cost 41*		**Cp/Cn**	**1.19**
	18	*Cost 24*		*Cost 42*		**Cp/Cn**	**1.20**
	19	*Cost 25*		*Cost 43*		**Cp/Cn**	**1.21**

Sum of Overhaul Costs			*Cost 44*	**£8,997.36**	

Present Value Life Cycle Cost (LCC) …	**£171,508.74**		
…of which:			
1 - Initial Investment Cost	**£38,492.00**	***Note***	
2 - Energy Cost	**£36,935.15**	***Note***	
3 - Planned Maintenance Cost	**£87,084.23**	***Note***	
4 - Overhaul Cost	**£8,997.36**	***Note***	